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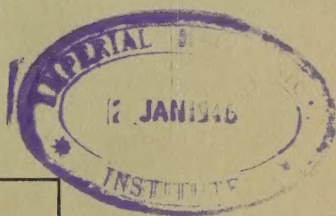
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PLANT FATS IN RELATION TO ENVIRONMENT AND EVOLUTION

JAMES B. McNAIR

INTRODUCTION

In the search for proper identification of plant groups and their true sequence in evolution, the chemical and physical properties of their fats may be useful. The following paper constitutes a review of the literature directly pertaining to this thesis. In favor of the use of fats in this connection is their wide distribution throughout the plant kingdom. One of the properties of fats which are useful in such identification is their ability to absorb iodine. The iodine value or iodine number of a fat or fatty acid is the quantity of iodine (in mg.) absorbed by one gram of fat or oil under specified conditions.

However, some properties of fats, including their iodine values, are affected by environment. The literature shows that the iodine absorption values may change in relation to the amounts of fatty acids present, although the kinds of fatty acids may not be changed by environment. Under similar environments fats may be separated according to their sequence in evolutionary position. In addition to the usefulness of the iodine value as an indicator of a plant group, there is the nature of the fatty acids themselves. For instance, some fatty acids are found only in certain plant families, *e.g.*, myristic acid in the Myristicaceae, erucic acid in the Cruciferae (38). Other properties which have been found useful in the identification of plant groups are increase in the number of carbon atoms in fatty acids and increase in the number of acids in seed fats with advance in evolutionary position (88).

The literature also shows that iodine values of fats give numerical values for plant groups, and by them more definite comparative values for evolutionary appreciation are determined than are obtained by other classification methods, *e.g.*, serum diagnosis. For instance, the iodine numbers of their fats are for palms (Principes

or Palmales), 29; for iris (*Liliiflorae* or *Iridales*), 81; for Rubiales, 111. These values indicate that palms are the most primitive, iris next, and the Rubiales most developed (87). The Rubiales may perhaps be considered as having developed four times as far as the palms, at least in their ability to produce unsaturated fats.

DEFINITIONS

Pure fats are solid or liquid oils consisting of a fatty (aliphatic) acid or a mixture of fatty acids chemically united to glycerine. Fats which are liquid at ordinary temperatures are also called fatty oils or fixed oils. Some fats are made up entirely or partially of unsaturated fatty acids. Unsaturated fatty acids are capable of uniting with certain other elements or compounds without elimination of any side product; thus, linoleic, linolenic and oleic acids are unsaturated. Unsaturated compounds have double or triple bonds; they are capable of uniting with iodine. Although it is possible for a fat to be made up of saturated fatty acids which do not combine with iodine, all known plant fats contain at least one unsaturated fatty acid.

FATTY OIL PRODUCTION IN RELATION TO STAGES OF DEVELOPMENT

According to the investigations of Ivanov (48), there occurs during the ripening of oil-seeds an increase of unsaturated constituents of their drying oils, whereas the composition of non-drying oils remains practically constant. If the oil of a ripe seed contains linoleic or linolenic acid, a gradual increase in the iodine number is observed in the course of development. Linseed oil in the initial stage of the ripening period gave an iodine number of 120, while the oil of the mature flax seed gave 180. Ivanov harvested flax at four stages of maturity. In 1910 the seed from the first harvest, one week after flowering, contained 4.4% of oil; the seed from the second harvest, on July 18, 13 days later, 11%; the seed from the third harvest, on August 3, 32.5%; and the seed from the fourth harvest, on August 25, 35%. These data show an extremely rapid rate of oil formation between July 18 and August 3, when practically all the oil was laid down in the seed.

Analysis of flax seed for oil content at four degrees of maturity by Eyre and Fisher (26) gave results which agree quite closely with those of Ivanov. They found that the maximum oil content

was reached and further production ceased with disappearance of the chlorophyll. The greatest rate of increase in the iodine value took place after the oil content had attained its maximum value. The value did not reach a maximum but rose throughout the whole period during which observations were made, for 53 days after flowering, although the rate of increase diminished somewhat toward the end of the period. Rapid oil formation occurred during the first 20 days when the color of the seed and oil denoted the presence of chlorophyll. After that period the seed assumed yellowish-brown and full brown and the oil a deep yellow, and the iodine value rose rapidly. The iodine value increased from 115 on the tenth day to 195 on the fifty-third day.

Bushey, Puhr and Hume (11) harvested flax planted on June 10 at five stages of maturity. The seed from the first harvest, on August 25, was very immature but contained 29.51% oil. The seed from the fourth harvest of September 21, which was fully mature, contained 36.84%. The iodine number of the extracted oil was lower at each of the successive five harvest dates. The maximum value was 181.5 at the first harvest and the minimum was 161.0 at the last harvest.

Dillman (18) has measured growth in volume and formation of oil in developing flax seed. The seed used for the study was of known age, obtained by tagging a large number of flowers and collecting the seed at frequent intervals of their development. It is evident that a study made by this method gives more accurately the rate of development of the seed, since the material used for each of the harvests is of uniform and known age. The results of Dillman's studies made in 1926 and 1927 at University Farm, St. Paul, Minnesota, and at Mandan, North Dakota, show that the growth in volume of the seed, as determined by measurements of length, width and thickness, is relatively rapid, reaching a maximum 12 to 14 days after flowering. Growth of the seed, as determined by the daily increase in dry weight, continued for 33 days and then remained constant to the end of the ripening period, 40 days after flowering. The most rapid formation of oil, based on percentage of oil in the dry seeds, began at about the seventh day after flowering and continued for 15 to 18 days. After the maximum percentage was reached, there was little or no significant change up to full maturity. The maximum percentage was reached somewhat before the maximum dry weight of the seed.

Johnson's (73) experiments with the Bison variety of flax showed the average oil content, studied at two-day intervals, to increase quite uniformly from five to 25 days after flowering, followed by a slight decrease to maturity. The iodine absorption number made at frequent periods on the seed from the three-year rotation suggests that the saturated fatty acids are formed first in the synthesis of oil, but are progressively transformed to acids with a greater degree of unsaturation. The iodine numbers increased rapidly from five to 17 days after flowering, remained fairly constant for a short period, then decreased slightly to complete maturity.

It has been shown that accumulation of oil in seeds does not set in actively during the very earliest stages in their development, and the work of Ivanov (48) suggests that there is a period of very intense oil formation which occurs about midway between blooming and final maturity of the seed. This raises the question as to the existence of a "critical period" in oil formation which would have an important bearing on the effects of external conditions on the quantity of oil produced. This question was investigated with soy beans and cotton seed (29). Samples were collected at different stages of maturity to serve more definite information on this point. The weight of the seed was also obtained in each case, so as to ascertain changes in the absolute as well as relative oil content. To arrest respiration promptly and thereby avoid changes in composition, the immature seeds were dried in an oven at 70° to 80° C. In the case of soy bean, five or six pods were picked from each of 100 to 125 plants. The material was grown at the Arlington Experiment Farm, Virginia, the Peking soy bean in 1910 and the others in 1912. The several pickings from each variety were taken from the same plants at intervals, but for obvious reasons the different pickings did not necessarily represent the actual growth made by the beans in the intervals covered. They were strictly comparable, however, as to the relation between the oil content and size of the seed at the several stages of maturity. In the case of the cotton seed, care was taken to obtain the same number of immature and mature bolls from each plant, and these were always taken from the same branch, about 12 plants being used for each pair of samples.

In all cases the relative weight of the seed rather than the date of harvesting is to be taken as the more nearly correct index of the

stages of development. The first pickings of soy bean were made when the seeds were exceedingly small, and the final pickings represented the fully matured seed. The results are definite and conclusive. Except for the period immediately after blooming and that directly preceding final maturity, there is throughout the development of the seed a gradual rather uniform gain in oil content as compared with the growth of the seed. There is no evidence of a definite "critical period" for the accumulation of oil during development of the seed. Considering only the percentage of oil, there is a very sharp increase during the first few weeks after blooming, and then only a slow gain until near the end of ripening. During the final stage of ripening there is a decrease both in the size of the seed and in the oil content. This phenomenon, which was observed also by Muntz (98), is probably due to continued respiratory activity after assimilation has ceased. In the case of cotton seed, the immature samples were taken when the green bolls had reached full size and had begun to show numerous brown spots. As in soy beans, increase in oil proceeds somewhat more rapidly than growth of the seed.

In other investigations of soy bean oil (16), two sets of ten plants each of pedigreed stock were chosen for analysis at different stages of maturity. About ten pods were picked from each plant. The first picking was made on August 23 (Wisconsin) when the seeds and pods were green; the second on September 8 when the pods were turning yellow; and the third on September 18 after the plants were fully mature and the seeds thoroughly dry.

Analyses of the seed from these two sets of plants are shown in Table IX of citation No. 16. The two sets check each other fairly well. It is significant that the iodine number increases quickly at the last stage of maturity, there being very little difference in the early stages.

One explanation which may be offered to account for the fact that the late high line has a higher iodine value than the early low line is that the quick maturity of the low line may possibly retard the complete development of the unsaturated oils, whereas the slower maturity of the high iodine line may provide better conditions for oil formation.

Besides flax seed, soy beans and cotton seed, other seeds have been analyzed in regard to their oil content in relation to the stage

of maturity. For instance, Ivanov (63), in an investigation of the oils from henbane and thornapple seed, found the maximum oil content at the middle stage of ripeness and that the acid number and iodine number decreased as ripening progressed. The iodine value of sunflower oil was found to remain almost constant during ripening (September 26 and November 15), but the saturated acids decreased steadily from 15.14% to 6.70%, linoleic from 74.74% to 64.91%, while oleic increased from 9.89% to 28.35%. After November 16 the composition of the oil remained practically uniform (6).

OIL INCREASE IN RELATION TO CARBOHYDRATE PERCENTAGE

Ivanov (48) noticed that as the percentage of oil increased in ripening flax seed the percentage of sugars decreased. This suggested conversion of sugars into oil during the oil-forming process. The change from carbohydrate to oil in seeds has been noticed by many investigators.

OIL CONTENT AS AFFECTED BY LENGTH OF GROWING PERIOD

Independently of any differences between early and late varieties as such, it might be expected that differences in oil content would be influenced by both character and length of the growing period. Several investigators have concluded that the length of the growing period is an important factor in determining the starch and protein content of wheat. In plants of determinate growth, in which all seeds are developed during approximately the same period, the effect of the length of the growing period on the oil content may be studied by making successive plantings at intervals, since many such species show a marked tendency to shorten the growing period when planted abnormally late. Mooers (97) has called attention to this tendency in soy beans, and others (29) have determined the oil content in seed of several varieties of this crop at intervals during spring and summer months.

The different plantings of soy beans showed marked variations in size of the beans and in their oil content, but there is no definite relationship between these characters and the date of planting. In other words, the character rather than the length of the season in which the seed is developed seems to be the important factor. Seed from the latest planting contain a somewhat lower percentage of oil

than others, but this relationship is not verified in other tests. These additional data all show lack of definite relationship between size and oil content of seed and length of the growing period. Garner, Allard and Foubert (29) found, besides the above, that there is a remarkable difference between varieties as to the shortening of the period required for maturing seed when planted late, *e.g.*, Buckshot and Medium Yellow varieties.

Bushey, Puhr and Hume (11), working with flax, showed, as did Garner, Allard and Foubert (29) with soy beans, that delayed planting has very little effect upon the oil content of the harvested seed. In each instance plantings were made at 15-day intervals over a two-month period beginning at the normal planting date for the crop used. Each of these studies was conducted for a single year. One variety of flax and four varieties of soy beans were included in the tests. T. E. Stoa has furnished data (unpublished) on the oil content of flax seed from several dates of planting. His studies, covering a seven-year period, show conclusively that delayed planting has only a very slight influence on the oil content of the seed.

In experiments with the effect of delayed planting on the oil content of flax seed, other analyses of seed obtained from plants sown at five successive ten-day intervals beginning on May 1 in 1929 and 1930 showed a very slight reduction in oil content with delayed planting in 1929, but no consistent differences in 1930. The iodine number of the oil determined from two varieties in 1930 indicated a slight decrease with delayed planting for Bison but no significant change for Redwing (73).

VEGETATIVE VIGOR VERSUS FAT CONTENT

Adams and Mounce (1) found a correlation between the degree of vegetative vigor and the concentration of fats and lipoids in cranberry leaves. In the cranberry, food is stored not only as starch but also as oil. In the leaves the starch seems to be transitory, and most of the stored food is in the form of oil, whereas in the stem large deposits of starch may be found. Chemical analyses of bog plants show that there is a definite correlation between the degree of vegetative vigor and the percentage of fats and lipoids in the leaf blades, the percentage of ether-soluble substances being lowest in the most vigorously vegetative plants. Leaf blades only were

analyzed from plants several years old. The amounts of ether extract on the basis of dry weight were as follows: strongly vegetative plants 10.57%, intermediate plants 12.42%, weakly vegetative plants 14.58%.

ENVIRONMENTAL INFLUENCES ON FATTY OIL PRODUCTION

Heredity versus Environment. The growth, development and composition of any individual plant depend on the combined action of heredity and environment. It follows that in any investigation of heredity and environment in relation to plant life such methods should be followed as will make it possible to distinguish between these two forces. The principle of clearly distinguishing between the effects of environment and those due to heredity is simple enough in theory, but in practice it is often extremely difficult to follow because of interrelations between the two forces which are not fully understood. In this way, non-inheritable variations in the progeny of relatively pure strains grown under what would seem to be uniform environmental conditions become especially apparent when we come to deal with quantitative differences such as are involved in a study of variable chemical composition as influenced by the factors of nutrition. In the experiments of Garner, Allard and Foubert on the nicotine content of tobacco, the oil content of seeds of various species and other quantitative characters, efforts to insure the greatest uniformity possible in environmental conditions have failed to effect anything like uniform composition in the progeny of individuals representing the purest strains available. Some of the strains of tobacco under study had been inbred for eight generations.

It follows that in dealing with quantitative differences in composition as influenced by environment, a sufficiently large number of individuals must be used to avoid misleading results due to variations which cannot be brought under control. Where conditions have made it impracticable to grow relatively large numbers of plants for study the alternative of repeating the experiment can be adopted. It is equally important to use caution in reaching generalizations based on results obtained with too small a number of species or varieties. In the course of their work on the oil content of seeds, Garner, Allard and Foubert frequently observed that in soy beans (29), different varieties are not always influenced in the

same manner by environment. In such plants as soy bean or cotton, therefore, as many varieties as practicable were included in the experiments. In selecting plants for study those that were of special importance because of the oil content of their seeds and which were otherwise adapted to the work in view were taken. So far, they have made use of cotton, soy bean, peanut and sunflower. Some varieties of soy bean may be grown under a very wide range of conditions, and for this reason this plant was largely used in their experiments—grown in a number of State experiment stations.

In some cases there were differences of more than 100% in the size of the seed, and also very large differences in the percentages of oil, when soy beans were grown in different localities. It is evident that environment as well as heredity may tremendously affect the size of the seed and the quantity of oil stored therein. It should be noted, however, that the behavior of the four varieties of soy bean was by no means the same when grown in different localities. There seems to be one exception to this observation, namely, that the conditions at Pullman, Washington, were such as to produce in each case abnormally small seed.

As cotton does not thrive in a cool climate it has not been practicable to study the development of oil in the seed under such a wide range of conditions as in soy bean. It can not be stated, therefore, whether the quantity of oil stored in the seed is subject to wide fluctuations, as has been noted in soy beans. Data of Garner *et al.* (29) show that in a three-year test all six varieties of cotton produced considerably heavier seed, containing a decidedly lower percentage of oil, when grown in the Piedmont section of northern Georgia than when grown in the Coastal Plain region of South Carolina. The increase in size of seed in northern Georgia was not entirely offset by the decrease in the percentage of oil, so that the actual quantity of oil stored in the seed produced under these conditions was somewhat greater than in those grown in the Coastal Plain region. There was also considerable yearly fluctuation in the oil content of the seed in both sections, owing to varying seasonal conditions. As already noted, the uniformity in behavior of all the varieties of cotton contrasts sharply with the varietal differences observed in soy beans. The average difference in oil content for the six varieties of cotton as grown in the two localities is greater than the varietal difference in either locality.

Soil (Media) and Fertilizers. In dealing with the responses of plants to differences in environment, it is frequently sought to differentiate between the effects of climate and those ascribed to the soil. Climate, or the average weather, as ordinarily understood, refers to conditions of the atmosphere, of which temperature and moisture are perhaps the most important as factors of nutrition. But the soil is likewise subject to variation in temperature and moisture, and there must be a tendency toward equilibrium in temperature and moisture between these two media in which the plant lives.

It is true that under any fixed weather, or climatic conditions, plants grown on contrasted soil types may show well defined differences in their development, but such relationships are subject to change with any change in climatic factors. There is ample evidence to show that differences in plant development observed on contrasted soil types during one season may be completely reversed in another season (29). It is also true that in extreme cases difference in climate may produce certain definite differences in plant development more or less independently of the soil type. Within ordinary ranges of soil and climatic differences, however, it is hardly possible to develop far reaching generalizations as to the specific effects of either independently of the other, for change of climate results in a change of soil conditions, and *vice versa*.

In spite of the above mentioned limitations which must apply in considering soil and climate as environmental factors, it seemed desirable to Garner *et al.* (29) to obtain data as to the influence of differences in soil type on the accumulation of oil in the seed as produced under varying seasonal conditions. In the experiments with cotton six representative Upland varieties were grown for three consecutive years (1909-1911) at Thompson Mills, Georgia, on two adjoining but contrasted types of soil, the original lots of seed being used for each year's planting. For convenience these soil types were designated as "red soil" and "gray soil," respectively. Both belong to the Cecil series and were in a good state of cultivation. The red soil is comparatively heavy, tenacious clay, while the gray soil is an open-textured sandy loam. As all the varieties were affected in a similar manner, the results are averages for the six.

Taking the average results for the three years, the red soil gave

only slightly heavier seed, with a somewhat smaller proportion of hulls than the gray soil, and there was practically no difference in the oil content. Each year the seed was heavier and contained a smaller proportion of hulls on the red soil than on the light soil, but the case is quite different as regards the oil content. In 1909 the oil content was considerably higher on the red soil than on the gray, while in 1910 these relations were reversed and in 1911 the differences practically disappeared. In other words, the comparative effects of the two soil types depend on the seasonal conditions, and it so happened that there was a balancing effect for the three years covered by the experiment.

The tests with soy beans included a wide range of soil types and climatic conditions, and the results as a whole emphasize the fact that the relative effects of different soil types are not specific and constant but depend largely on seasonal conditions, as was brought out in the experiment with cotton. The results in the field experiment at Arlington Farm, as compared with those obtained in the greenhouse with the same soils, illustrate this point. In the field tests the plants suffered considerably from drought during the growing season, and the sandy soil gave decidedly smaller beans and higher relative oil content than the clay soil. In the greenhouse the difference in size of beans largely disappeared, while the clay loam gave a somewhat higher percentage of oil than the sandy soil. In the pot experiments at Arlington Farm the lighter soils gave somewhat larger seed with lower percentage of oil than the heavier clay soil, but at Manning, South Carolina, there were no significant differences.

Experiments similar to those with soy beans were made with the Spanish variety of peanut. The effects produced by the different soil types are of the same general character as with soy beans, although the behavior of the two species under similar conditions is not always the same. In 1913 a series of pot cultures with sunflower were carried out at the Arlington Farm (29) in the same manner as for soy beans, using a number of different soils in the test. Soil differences brought about very marked differences in the size of the seed, but the variations in relative oil content were less decided.

On comparing the data of oil content of soy beans grown under different environmental conditions with related data obtained

with different soil types it becomes apparent that variations in the size of seed and the oil content of soy beans attributable to differences in soil type are far less than those observed when both soil and climate differ. The same relationships are observed in cotton seed. These results are interpreted as indicating that under practical conditions climate is a more potent factor than the soil in modifying the size of seed and its oil content. The most probable explanation is that the atmosphere is subject to greater and more rapid variations in moisture and particularly in temperature, and also that the "soil climate" is greatly influenced by the weather conditions. Temperature and moisture differences of both soil and atmosphere are among the important factors of environment which may influence the plant characters under study, and this factor complex must be at least partially analyzed before satisfactory conclusions can be reached as to the principal external factors concerned in oil formation in the plant.

Bacteria. A colorless strain of *Serratia marcescens* was grown on agar and the lipid content determined (31). On meat extract at pH 7 the lipid content reached 0.6% (dry basis) in eight days. With increasing age the lipid fell. Age and pH are so related that the lipid content remains nearly constant near neutrality but decreases at acid or alkaline reactions. On fluid media of the same composition two to four times as much lipid is formed.

Mold. Single cell cultures of *Aspergillus fischeri*, grown on synthetic media containing glucose as the sole source of carbon, showed differences in the amount and nature of the fat, depending on the initial concentration of glucose, concentration of ammonium nitrate, pH, temperature, increased aeration and period of incubation (104). A high fat content was favored by neutral or slightly alkaline media, a high concentration of glucose and a low concentration of ammonium nitrate. The percentage of sterol in the mycelium was increased by the use of a fairly high initial concentration of glucose, 1.0% of ammonium nitrate or an initially acid medium containing urea, a higher temperature (37° C.) and a long period of incubation. The percentage of lipinphosphorus in the mycelium was increased by the use of a low initial concentration of glucose, 1.0% of ammonium nitrate and an initially slightly acid medium.

Fertilizers. The experiments in different soil types with soy beans, cotton, *etc.*, previously described, have included soils varying greatly in fertility, as indicated by the comparative growth of the plants, and the results as a whole show that within the limits ordinarily met in farm practice the relative fertility of the soil does not greatly influence the size of the seeds or oil content (29). A large number of fertilizer tests with cotton were carried out at Lamar and Timmons ville, South Carolina, in 1909, 1910 and 1911, to obtain more accurate information as to the effects of fertilizers on the size and oil content of seed. The results are averages of duplicate plots, except for the controls, which represent the averages for four plots in each case, all plots being 1/40 acre. The tests in 1909 and 1910 included plots receiving four different quantities of nitrogen, four of phosphoric acid, and three of potassium. Dried blood, acid phosphate and muriate of potash were used as fertilizers.

The soil used in 1911 was very poor, as shown by the large increases in crop yields produced by the complete fertilizers. Addition of all three elements, nitrogen, phosphorus and potassium combined in various proportions, gave in all cases considerably heavier seeds, with a smaller percentage of hulls and a higher oil content in the kernels as compared with the controls. With respect to the varying quantities of the three fertilizer elements, increased applications of nitrogen had no appreciable effect on the weight of the seed and only a slight effect on the percentage of hulls, but lowered considerably the oil content of the kernels. Increased applications of phosphorus and potassium did not materially affect any of these characters. The tests of the two preceding years gave similar results.

Pot culture tests were made in 1911 with the Peking variety of soy beans, using tile cylinders filled with Arlington clay soil. In a series in which phosphorus and potassium in a fixed ratio were added in three different quantities, the yields of beans were greatly increased and the weight of the seed was not changed, while the oil content was increased about 20%. With addition of phosphorus alone, very much the same results were obtained; but with addition of potassium alone there was only a small increase in yield and practically no increase in oil content. In similar tests with Spanish peanuts phosphorus gave a large increase in yield

and slightly increased the weight of the peas, but had no effect on the oil content. Potassium had practically no effect on the yield, the weight of seed, or the oil content.

In his study of fat production in *Picea excelsa* and *P. sitchensis* seedlings grown in cultures, Laing (77) found in both species under all treatments that fats were in the leaf, except where potassium was omitted from the culture. But there was a stronger reaction for fats in the root system of plants in potassium-free cultures than in the presence of potassium. Fats were absent from the root tissues of *P. excelsa* when phosphorus was omitted from the culture.

Linseed oil was found to have a somewhat higher iodine number when produced on non-fertilized fields than when produced on fertilized fields. They were, respectively, 189.7 and 188.1 (7).

Temperature. The temperature at which fats are formed is one of the factors which determine their degree of unsaturation. This is based upon three separate lines of reasoning (79). First, a general survey of the fats in animals and plants indicates that the highly unsaturated fats are most commonly found in plants growing in temperate or cold regions (103, 52, 83) and in the poikilothermic animals which live under relatively cold conditions, while the most saturated fats are found in tropical or subtropical plants and in warm blooded animals. The carnivora should not be included in this generalization because the composition of their adipose tissue fat is partly, at any rate, determined by the fat which they take in as food, and even in animals, such as the pig, which produce fat easily from carbohydrate, differences have been observed in the composition of their stored fat which may be referred to differences in the composition of the fats present in the cereals on which they have been fed. Secondly, it is usual to find that the fat in living organisms is fluid at the temperature at which the organisms exist, except endosperm fats. This would preclude the formation of saturated acids to any great extent in organisms living under temperate or cold conditions, because such acids would tend to raise the melting points even of mixed glycerides which contained them. The higher melting points of the saturated acids would be less important from this point of view in the warm blooded animals or tropical plants, and it might be more likely, therefore, to expect saturated acids to occur to a

greater extent in organisms living under warmer conditions. Thirdly, various hypotheses have been made regarding the chemical reactions by which fatty acids are formed in nature, namely, autocondensation of acetaldehyde (78), condensation of pyruvic acid and acetaldehyde (111) or autocondensation of pyruvic acid (99). All these admit of the possibility of formation of long chains of carbon atoms with unsaturated linkages as products of the condensation reactions which produce them, and if they are to give rise to saturated carbon atom chains a reduction must take place subsequently. If this represents what takes place, then the unsaturated acids would be precursors of the saturated. Two different types of reaction are involved, namely, condensation and reduction. It seems possible that both these reactions would not necessarily take place with the same ease at low temperatures, and, if it be assumed that the condensation takes place readily at low temperatures whereas reduction requires higher temperature, an explanation of the distribution in nature of the saturated and unsaturated acids referred to above might be found on these lines.

In experiments with fungi it has been noted that the more unsaturated fats are produced at a lower temperature. By comparing the energy loss from the medium during growth with the potential energy of the fungus formed at different temperatures, the expenditure of energy was somewhat greater at the higher temperatures (116). This indicated that production of the more saturated acids under these conditions involves greater utilization of the potential energy of the medium and suggests that reduction succeeds condensation in the series of reactions by which fatty acids are produced, and thereby involves greater expenditure of energy. However, according to others (63), lower saturated fatty acids are first formed and the higher unsaturated acids later. This view is supported by still others (107, 25, 6).

Mild warm climate favors the formation of saturated fatty acids and of unsaturated acids with one double bond. Cold climate favors formation of unsaturated acids with two or three double bonds (56).

Linolenic acid, $C_{18}H_{30}O_2$, is the most sensitive to climatic conditions (52), the reason being that this acid with its three double linkages is easily oxidized and readily absorbed by plants where it develops heat. The action appears whenever plants are obliged

to accommodate themselves to cold climates, and may date back to the conifers at the end of the Paleozoic age. Linolenic acid is said to be the first vegetable product met with which permits an analysis of "accommodation" from the chemical point of view.

Oils containing glycerides of unsaturated acids with one double bond (oleic, erucic or ricinic) were indifferent toward climatic changes (57), while with acids having three double bonds (α - and β -linolenic acids) the iodine number decreased with increase in temperature or with the growth of the plant in more southerly regions. Oils with two double bonds (of the linoleic acid type) held an intermediate position.

Pigulevski (102, 103) found that a plant growing in a cold climate produces fatty acids of a lower degree of saturation and therefore of greater chemical activity than the same plant growing in a warm climate.

Ladd (76) arrived at similar results in experiments with soy beans. Soy plants from warm regions that gave an oil having a rather low iodine number were later transferred to colder localities. They then produced seeds whose oils gave higher iodine values. Conversely, a lowering of iodine numbers was obtained from oils from such seeds which had been shifted from north to south.

Low temperature (20° C.) was found to increase the iodine values of the fatty acids produced by the mold *Aspergillus fischeri* (104). The iodine value was 93 at 20° C. and 88 at 37° C. The iodine values were likewise increased when the mold was grown on a low concentration of glucose (1.0 to 70.0 gms. per 100 ml. of solution) or on a medium which became strongly acid (e.g., NH_4Cl as nitrogen source). The neutral equivalents of the fatty acids were in all cases very near to 280, suggesting a preponderance of fatty acids which contain 18 carbon atoms.

Tuttle (118) examined a number of trees, shrubs and perennial herbaceous plants of the region near Edmonton, Canada. All species examined showed a high starch content during summer, which disappeared in October. With the exception of *Lonicera glaucescens* and *Crataegus* sp. all the trees and shrubs contained oils and fats as food reserve during winter. The other genera examined were *Syringa*, *Populus*, *Prunus*, *Salix*, *Shepherdia*, *Ribes*, *Picea*, *Pinus*, *Rosa*, *Pyrola*, *Cornus* and *Eleagnus*. In his

study of the evergreen leaves of *Linnaea* (117) he found starch formed at moderately elevated temperatures and that such starch is converted into oil when the temperature is gradually lowered. The oil is reconverted into starch when the temperature is again raised.

In a study of the occurrence of starch and oil in seeds it was noted that starch was present less frequently in the seeds of temperate plant families than in those of tropical plant families (85).

We have in the so-called bioclimatic law of latitude, longitude and altitude an example of a natural law which represents the general laws of climate as affecting the seasonal activities of plants. In the United States this variation, other things being equal, is at the rate of four days for each degree of latitude, 5° of longitude and 400 ft. of altitude (41, 42). We can comprehend the effect of this law on both qualitative and quantitative fat formation.

Latitude. As plants grow nearer to the equator the calorific value and the iodine number of their fats decrease (50, 52, 53, 55, 64). This is shown in Table I.

When plants are transplanted from a northern climate to a southern or *vice versa*, the oil content changes to correspond with the climate (76, 55, 52, 53).

Oil from the nuts of *Pinus cembra* of western Siberia was found to consist of oleic, linoleic and linolenic glycerides. Of these the content of linolenic acid was highest in the most northern climate (66).

The seed fats of seven species of *Paeonia* showed increasing iodine values in going from south to north and from sea level to higher altitudes (69, Table I). These plants grow wild in the Siberian forests, in Altai, Ural, Caucasus and in the Crimea. They produce a great many seeds which contain from 26% to 41% of fat.

In coconut palm oil the unsaturated fatty acids increased from south to north and the iodine value likewise (62). The iodine value at the equator is 4 to 10 for *Cocos bonetti*, in Suchum it is 23.6.

In a study of the fatty oils of the Labiatae and Anacardiaceae (64), the content of fatty acids with three double bonds was found to diminish as one goes from the northern countries toward the tropics.

TABLE I
INFLUENCE OF LATITUDE AND ALTITUDE ON THE IODINE VALUE OF PLANT OILS

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
<i>Iris ruthenica</i> (Iridaceae)	Shebalino (Altai)	50-52°	800	106.2 (33)
	Katum River	1200	108.1 (33)
	Seminski Pass	51°	1860	117-119 (33)
<i>Paeonia anomala</i> (Ranunculaceae)	Seminski Pass (Altai)	50-52°	800	31.7	142.2 (69)
	Gov. Perina	41.1	144.4-145.8 (69)
<i>Paeonia anomala</i> var. Beresovsky	Seminski Pass (Altai)	50-52°	1870	32.4	157 (69)
<i>Paeonia tenuifolia</i>	Leningrad	60°	24.5	153.5-155.2 (69)
	Crimea, Karadag	34.0	145.8 (69)
	Moscow	55°50'	29.7	148.8 (69)
	Karadag	28.7	127.7-128.9 (69)
<i>Paeonia corallina</i>	Sympheropol	26.3	130.3 (69)
var. <i>triflornata</i>	Leningrad	60°	24.6	138.7 (69)
"	Sibirsk	54°	about 50	134.5 (58)
<i>Trollius asiaticus</i> (Ranunculaceae)	Seminski Pass	51°	1860	149 (58)
	Tifis	41°40'	134-138.5 (58)
<i>Glacium luteum</i> (Papaveraceae)	Moscow	50°50'	145.9 (58)
	Turkestan	41°	144.4 (58)
<i>Roemeria rhoeadiflora</i> (Papaveraceae)	Moscow	55°50'	157.7 (58)
	Erfurt, Germany	51°	103.6 (67)
<i>Thlaspi arvense</i> (Cruciferae)	Rostov (Donu)	47°10'	about 50	110.5 (67)
	Bakuriani	41°25'	1670	128.9 (67)
	Ust-Tsyla	65°10'	134.2 (67)

TABLE I—(Continued)

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
<i>Erica sativa</i> (Cruciferae)	Tashkent	41°	96-97 (33)
	Samarkand	40°	97.4-98.1 (58)
	Moscow	55°50'	98-99 (33)
	Moscow	55°50'	100-101 (58)
<i>Brassica campestris</i> (Cruciferae)	Mindau, Turkestan	33.0	95-102.5 (67)
	Cuba	45°	94 (58)
	Moscow	55°50'	96 (58)
	Transbalkans	84°	300	98 (58)
	Biisk	52°	100.4 (67)
	Moscow	56°	101.0 (67)
	Transbalkans	104.3 (33)
<i>Brassica rugosa</i> (Cruciferae)	Moscow	55°50'	104.4 (33)
	Buitenzorg	7° So. Lat.	89.1 (67)
	Rostov (Donu)	47°	98.1 (67)
<i>Raphanus sativus</i> (Cruciferae)	Prain in Biisk (Altai)	52°	100.4 (67)
	Buitenzorg, Java	7° So. Lat.	98.1 (67)
<i>Capsella bursa pastoris</i> (Cruciferae)	Biisk	52°	114 (67)
	Poltava	49°30'	128.14 (67)
<i>Camelina sativa</i> (Cruciferae)	Novgorod	59°	138-139.3 (67)
	Karadag (Crimea)	45°	133.12 (67)
	Poltava	49°30'	140 (33)
	Tambov	53°	149.8 (67)
	Moscow	55°50'	154.1 (33)

TABLE I—(Continued)

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
<i>Neslea paniculata</i> (Cruciferae)	Rostov (Donu)	47°10'	about 50	130 (58)
	Bakuriani	41°25'	1670	133.5 (67)
	Bakuriani	41°25'	1670	139.5 (58)
	Moscow	55°50'	about 190	141 (67)
<i>Hesperis matronalis</i> (Cruciferae)	Bakuriani	41°25'	1670	148.2 (67)
	Moscow	56°	149–155.3 (67)
<i>Linum usitatissimum</i> (Linaceae)	Tashkent (Central Asia)	41°	154–158 (58)
	Tiflis	41°40'	sea level	154–160 (33)
	Tiflis	41°25'	154–164 (33)
	Kursk Tula	36°–37°	174–176 (58)
	Bukuriani	41°45'	179.4 (58)
	Moscow	55°59'	1670	176–184 (58)
	Biisk-Barnaul	85°	185–194 (58)
	Archangel	65°	195–200 (58)
	Java	7° So. Lat.	85–86 (58)
<i>Ricinus communis</i> (Euphorbiaceae)	Persia	35°	84–87 (58)
	Turkestan	41°	86 (33)
	North Caucasus	45°	88 (33)
	Moscow	55°50'	87–88 (58)
<i>Althaea ficiflora</i> (Malvaceae)	Tashkent	41°	116.1 (33)
	Moscow	55°50'	119.4 (33)

TABLE I—(Continued)

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
<i>Olea europaea</i> (Oleaceae)	North Africa	29°	84-89 (58)
	Suchum	43°	85 (58)
	Italy	43°	84-89 (58)
	Tashkent	41°	120.3-122 (33)
<i>Luffa acutangula</i> (Cucurbitaceae)	Buitenzorg	7° So. Lat.	133.3 (33)
	Palermo	38°	138.2 (33)
<i>Ecballium Elaterium</i> (Cucurbitaceae)	Tashkent	41°	144.9 (33)
	Buitenzorg, Java	7° So. Lat.	115.1 (33)
<i>Cucumis sativa</i> (Cucurbitaceae)	Moscow	55°50'	132 (33)
	Saratov	51°40'	122.4 (33)
<i>Cucumis citrullus</i> (Cucurbitaceae)	Biisk (Altai)	52°	127.7 (33)
	Central Africa	24.7	115.1 (68)
<i>Carthamus tinctorius</i> (Compositae)	Tashkent	41°	30-32	124.2 (68)
	Ashabad	38°	118 (58)
<i>Helianthus annuus</i> (Compositae)	Voronezh	51°40'	126-130 (58)
	Omsk	55°	140.4 (58)
	Central Africa	81.5 (68)
<i>Madia sativa</i> (Compositae)	Poltava	49°30'	117-134 (68)

In a study of the fatty oils of the Cruciferae and Compositae (67, 68), the iodine values of the oils of both families increased from southern to northern locations and from lower to higher altitudes. The least variation was in oils which contained fatty acids with one double bond, and the greatest variation in acids with triple bonds.

The percentage of oil in different varieties of flax seed was found by N. N. Ivanov (45) to remain nearly constant in various climates (arctic, subtropical, maritime and continental). He considers the oil content in these plants to be an inherited property. However, the iodine value of the oil was found to change. It increased when plantings were shifted to the north. S. S. Ivanov concluded from his work on flax seed in 1932 that this seed, when produced at high temperatures, formed negligible amounts of linolenic acid. On the other hand, in northern or alpine climates large amounts of unsaturated fatty acids are formed. This corroborates the general rule that warm climates favor formation of oleic acid, while northern climates favor that of linolenic acid. Linseed from Nalinsk (Russia) was cultivated at two stations in Switzerland, at Liebefeld, 550 meters altitude, and at Davos, 1550 meters altitude; also in the tropical house of the botanical gardens at Berlin. The iodine numbers (Hübl) of these oils were: Nalinsk 185.1, Liebefeld 188.4, Davos 189.6, Berlin tropical house 92.57. The weather at the Swiss stations was rainy and cold, whereas the temperature at the Berlin house was kept at 25°-30° with an atmosphere saturated with water vapor.

Fachini and Dorta (27) found that olive oils from the northern parts of Italy (Lake Garda, Liguria) contain only small proportions (2% to 6%) of linoleic acid, whereas certain oils from southern Italy, Greece and northern Africa contain considerable quantities, even as much as 17%, together with a high percentage of saturated fatty acids. These authors maintain that the composition of olive oils seems to be influenced by the age and degree of acclimatization of the plant. The increase in the percentage of linoleic acid with approach to the equator is the opposite observation to that usually found. However, the increase in the amounts of saturated acids near the equator is in harmony with the general rule, and perhaps these so dilute the unsaturated acids present that the iodine value for the southern oil is less than that of the northern.

Altitude. Analyses of petroleum-ether extracts of the wood of *Cedrus deodora* growing in the Himalayas gave an increase in the iodine value (Hübl) from sapwood to heartwood and with increase in altitude. All samples were taken in January and gave the following results: 5000 ft. sapwood 101.3, heartwood 101.9; 7000 ft. sapwood 101.4, heartwood 102.2; 9000 ft. sapwood 101.6, heartwood 102.9; 10,000 ft. sapwood 109.7, heartwood 113.0 (92, 93).

Increase in altitude has been found to increase the iodine number of seed fats in many plants (Table I). Altitude increase, latitude increase and temperature decrease have all been found to increase the iodine number, and *vice versa*.

Submergence. Haas and Hill (34), in their study of some British brown algae selected with regard to their vertical distribution, showed an increase in fat and fat-like substances with the degree of emergence accompanied by an increase in the saturation of these substances and an increase in unsaponifiable residue with the depth of immersion.

The Phaeophyceae of Britain are zoned from the sublittoral through the littoral to the salt-marsh region, and the conditions of life vary accordingly. At the lowest limit, the laminarias are emersed only at the neap spring tides; consequently, they are exposed to the air for only short periods, and run but small risk of desiccation; they are subject to a narrow range of temperature and to a wide range in light intensity. Thus, for the most part, they live in serene circumstances, almost a thalassic Avilion.

At the highest limit, fucoids of the salt marsh are submerged only at high tides, maybe only by the spring tides, *Pelvetia canaliculata* f. *libera*, for example. During their long exposure they may experience wide ranges in temperature, and the coincidences of weather conditions may produce such periods of drought that the plants may become almost brittle as a result of desiccation. Their existence is subaerial rather than aquatic.

The conditions of life in the littoral zone vary between these extremes, and need no further comment.

The Rhodophyceae, although for the most part sublittoral, also show some zonation: *Polysiphonia fastigata*, e.g., often is epiphytic on *Ascophyllum* and *Bostrychia scorpioides*, a salt-marsh plant, often growing in association with *Pelvetia canaliculata* f. *libera*.

Contemplation of these extremes lead to many questions, especially concerning the correlation between metabolism and habitat.

The following table shows various fucoids arranged, as nearly as may be, in order of zonation downwards. For the sake of comparison, two members of the Rhodophyceae, which normally occur at the extremes of zonation, are added, together with two of the Chlorophyceae which were collected at the highest tide levels. The ether extract consists of fat and fat-soluble substances, including pigments. The results for the most part are for gatherings made at the same season, although not necessarily, for obvious reasons, from the same locality:

PLANT	ETHER EXTRACT %
<i>Pelvetia canaliculata</i> f. <i>libera</i>	8.62
<i>Pelvetia canaliculata</i>	4.88
<i>Fucus vesiculosus</i> f. <i>volubilis</i>	3.76
<i>Ascophyllum nodosum</i>	2.87
<i>Fucus vesiculosus</i>	2.60
<i>Halydria siliquosa</i>	2.18
<i>Himanthalia lorea</i>	1.21
<i>Desmarestia aculeata</i>	0.65
<i>Laminaria digitata</i>	0.46
<i>Bostrychia scorpioides</i>	0.31
<i>Chondrus crispus</i>	0.204
<i>Enteromorpha intestinalis</i>	0.217
<i>Ulva latissima</i>	0.185

The amount of ether extract detected in *Laminaria* corresponds quite closely to that obtained by other investigators. For instance, König and Bettels (75) found 0.39% to 0.5% in air-dried material, and Hoagland (40), in his work on the kelps of the Pacific coast, gives the following percentages in terms of dry weight:

PLANT	ETHER EXTRACT %
<i>Laminaria Andersonii</i>	0.65
<i>Macrocystis pyrifera</i>	0.34-0.40
<i>Nereocystis Luetkeana</i>	1.06
<i>Pelagophycus porra</i>	0.27

Some seasonal variation occurs, however, as is shown by the following:

<i>Pelvetia canaliculata</i>	October	4.88
	May	5.84
<i>Laminaria digitata</i>	March	0.46
	July	1.36

Such variation, however, was not noted in *P. canaliculata* f. *libera*, the average value for October being 8.62% and for May 8.65%. This is hardly surprising, for the dominant factor in its life is periodic emersion, generally for relatively long periods.

QUANTITATIVE FAT AND IODINE VALUE

	1	2	3	4	5	6
	Ether extract	True fat	Un- sapon. of 1	Fatty acid of 1	Iodine value of 1	Iodine value of 4
<i>Pelvetia canaliculata</i> f. <i>libera</i> ..	8.0	6.2	7.6	72.5	106	107
<i>Pelvetia canaliculata</i>	4.9	3.6	10.8	69.9	115	124
<i>Fucus vesiculosus</i>	2.6	1.9	16.9	71.6	114	108
<i>Laminaria digitata</i>		0.16	25.9	49.9	123	110

These figures relating to the Phaeophyceae are of considerable interest in that they show a direct correlation between the amount of fat and vertical distribution of the weeds, *i.e.*, duration of exposure. The greater the duration of emersion the greater is the exposure to conditions favoring desiccation and to a wider range of temperature. Here there is a remarkable parallel with what may occur in land plants, for the chemical nature of the reserve food in many evergreen plants varies with climatic conditions, and fat and fat-like substances may appear in the leaves on the advent of winter, often at the expense of starch (117, 118, 94, 20).

The iodine value of the ether extract of the Phaeophyceae falls with the degree of emersion, being highest in *Laminaria* and lowest in *P. canaliculata* f. *libera*, which means that the fat and fat-like substances of the latter plant are the more highly saturated. This may be correlated with the more extreme conditions of its life, especially a higher temperature for the most part of the year and prolonged periods of desiccation.

It is to be pointed out, however, that the iodine values of *P. canaliculata* and *F. vesiculosus* are practically identical, but the measure of their vertical separation is not more than a few feet.

The relation between the degree of saturation and vertical distribution would not appear to obtain when the iodine values of the fatty acids of the selected plants are compared. It is uncertain what significance can be attached to these figures, for during isolation of the fatty acids from the original ether extract they became more saturated, although reasonable precautions were taken.

For the rest, it may be mentioned that there is a greater propor-

tion of liquid fatty acids in *P. canaliculata* f. *libera* than in *L. digitata*.

These observations apply only to the Phaeophyceae. The two forms of Rhodophyceae examined, *Bostrychia* and *Chondrus*, representing the extremes of the habitat, contain but little fat, and the disparity between the amounts is not so great as in representatives of the Phaeophyceae. Similarly, the two great algae, *Enteromorpha* and *Ulva*, characteristic of the upper tidal reaches, contain but little fat, and there is no corresponding plant of the lower tidal limits with which to institute a comparison.

Shade. Slight shading was found to increase the starch content of flax seeds, while the oil content was lowered. The quality of the oil was not influenced, but with heavy shade the iodine number was below normal (50).

Moisture. According to Antevs (3), the temperature and water content of trees are the most important factors in determining the ratio between reserve substances. He found that starch solution and fat formation during winter are more pronounced in the same species in Stockholm than in middle Europe. Fat solution and starch regeneration in spring depend to a great extent on climate. "Fat trees", e.g., *Alnus*, can exist during winter without the type of fat which gives a reaction with Sudan III, and also without starch. Instead they possess an unknown fatty substance, colored yellow by Sudan III. Some "fat trees" (*Salix caprea* and *Prunus padus*) contain this unknown fat in moderately large quantities, besides typical fat and starch. Contemporary with starch regeneration the unknown fat is transformed into typical fat and starch.

Sinnott (108) suggests that the type of food reserve may be due to differences in the water content of the storage cells giving a modification of enzyme action or to differences in the ease with which enzymes have effective access to the storage cells. Where the movement of liquids is slow, starch predominates, and where it is easy, starch disappears at the beginning of winter and fat is produced. From a study of the distribution of starch and fat in woody plants, Sinnott concludes that during winter starch is commonest in regions remote from centers of production, and in cells with thick lignified, small pitted walls, and that fat is most abundant in and near the phloem, close to the vessels, and in cells with thin or unlignified walls or large pits.

Although the oil content of the same plant generally decreases from northerly to southerly latitudes, increasing moisture content of the soil has been found to increase with the total oil in the seeds (47). In flax the iodine number of the oil increases with the moisture content of the soil. In the irrigated southern districts of Russia the oil content of flax reaches 40% and the iodine number is 181.

In the 1933 crop of Canadian flax seed Geddes (30) found the iodine number of the oil somewhat lower than those of previous crops from the same localities. This, he thinks, may have been caused by a deficiency of soil moisture, which would tend to increase the ratio of oleic to linoleic and linolenic acids and deleteriously affect the drying quality of the oil.

The percentage of oil in oil-bearing plants grown under irrigation was found to increase up to a certain number of irrigations, beyond which it decreased (46). The iodine number did not increase to an appreciable extent with increase in the number of irrigations except in flax.

A study of the maturing seed of *Linnaea* and hemp showed the quantity of oil to increase to a certain maximum as the seed became dry (19). In immature seed, dried after shelling, the amount of oil is not increased because normal maturation is arrested. Under ordinary conditions of germination the amount of oil is diminished to one-half after two hours of swelling and is negligible after 24 hours. If the presence of oxygen hinders germination, the quantity of oil remains the same, although the grains have absorbed enough water. In normal conditions of germination water is indispensable for transformation of oil. In winter the amount of oil in birch and linden trees is increased and at the same time water is diminished. In spring, with absorption of water, the amount of oil is decreased.

As in many higher plants, so in yeast, the equilibrium carbohydrate \rightleftharpoons fat is controlled by variations in water content. When cultivated under conditions which kept the water below 85%, yeast showed an enormous increase in fat and sterol content (35). The total lipoid can thus be increased 20 times and the sterol 60 times the original values.

On solid media, *e.g.*, nutrient agar, the sterols increase more rapidly than the glycerides, but acidity rapidly increases also.

The low water content required for the fat and sterol enrichment is obtained by pressing the yeast and spreading it on porous plates.

Spacing. Dense sowing of flax was found to increase the iodine number of the oil to an average of 189.9, while thin sowing lowered the average to 187.9 (7). Fomin (28) noticed that variation in the spacing of sunflower plants had considerable influence on the oil content of their seeds.

HEREDITARY VARIETAL DIFFERENCES IN FATTY OIL CONTENT

Except for experiments on plant pigments and considerable practical breeding for sugar in beets, there has been little direct research on the inheritance of chemical characters in plants.

Corn—oil percentage. The Illinois corn breeding experiments (112) on selection for high and low protein and high and low oil content stand preeminent in this field. Starting with a variety of average composition, it was possible by selection and breeding in ten generations to increase the average oil content from 4.70% to 7.37% and to decrease it from 4.70% to 2.66%. These results were conclusively shown to be quite independent of soil, climate or cultural conditions.

By analyzing the pedigree records of the Illinois experiment, Surface (115) was able to show that the selection process had merely isolated already existing types of oil and protein percentage, the intermediate types having been discarded during the years of selection. This process is characteristic of an open-pollinated crop like corn.

Pearl and Bartlett (100) describe a preliminary undertaking on the heredity of chemical characters in maize. From their experiments they infer that the seed characters with respect to moisture, nitrogen, protein, crude fat, ash, crude fiber, pentosans, sucrose, dextrose and starch are inherited in maize essentially in accordance with Mendelian principles. They state that "probably each of the characters, protein, crude fat, and ash content, segregates as a definite and distinct unit character".

Lindstrom and Gerhardt (80) found that dent and sweet varieties of corn were sharply differentiated in the chemical constitution of their seed, especially in relative amounts of sugars, dextrans, starches and fat. This fact, together with our knowledge

of the mechanism of heredity in this species, provides an excellent background for an exact determination of the mode of inheritance of these chemical characters. Another advantage in the genetic analysis lies in the precise, cellular mechanism of double fertilization in the embryo sac whereby the endosperm tissue is developed as the result of a triple fusion of nuclei, two from the female and one from the male parent. This nuclear behavior permits a detailed comparison of the influence of one, two or three doses of genetic factors for any endosperm characters, including the chemical characters involved in these investigations. A thorough genetic study of endosperm factors controlling aleurone and endosperm color has demonstrated beyond reasonable doubt that their inheritance is typically Mendelian in nature. It becomes of immediate interest to determine whether chemical characters, from the quantitative standpoint, obey the rules of inheritance as do the qualitative characters of the endosperms noted above.

In the original or parental types used (80), sweet corn had nearly twice as much fat as dent corn when the entire kernel was used for analysis. When only the endosperm was used, this same sweet corn had nearly three times as much fat. This, of course, indicates that in dent corn most but not all the fat resides in the embryo, whereas in sweet corn a much larger proportion actually exists in the endosperm itself. This relation is also consistently shown in the various hybrid generations of this cross. Calculations show that approximately 74% of the total fat content in dent corn resides in the embryo, the remainder presumably being in the endosperm tissue, particularly in the aleurone layer of cells.

In sweet corn kernels, more of the fat was contained in the endosperm tissue. In these kernels approximately 64% of the fat was in the germ, leaving 36% for the endosperm and pericarp tissues.

The F_1 kernels of the sweet corn \times dent corn cross distinctly show an intermediate condition with a slight dominance of the lower fat percentage, which is consistently true whether the entire kernel or only the endosperm tissue be considered. Pearl and Bartlett (100) found the same general situation in their crossbred or F_1 kernels, using only the entire kernel for analysis.

Segregation of high and low fat values in the F_2 and backcross generations is very evident. The extracted sweet corn in all cases

is practically as high or higher in fat than in the original variety. This is true both in the entire kernel and in the germless grains. Presumably there is a direct association of fat values with the carbohydrate situation. There is, however, no apparent cumulative effect in fat values which might be expected if this association were complete.

In all the crosses reported in these investigations, the parental varieties of dent or sweet corn carried different endosperm colors, one or the other being yellow. Because of this relation it is possible to determine any association of endosperm color with high or low sugar, starch or fat content, whether this association be due to genetic linkage or to chemical or physiological correlation. Pearl and Bartlett's (100) data suggested a slight correlation between these characters. In their F_2 generation from a Yellow dent \times White sweet corn cross, there seemed to be a higher sugar content in the yellow F_2 seeds than in the white. Conversely the white seeds showed a higher fat value. This finding was confirmed by Lindstrom and Gerhardt and was traced to a genetic linkage.

Soy beans—oil percentage. Varieties of soy bean tested at the Delaware Agricultural Experiment Station showed considerable range in time of maturity, size of plant, coarseness or fineness of plant parts, and yield of seed (32). Some varieties stand erect, others have the tips of the branches slightly twining. Varieties exhibit considerable differences in the habit of bearing their branches near the ground. The color of seeds may be black, yellow, olive, green, brown or mottled. Size of the seed also varies widely. In some varieties 35 seeds will weigh 10 grams, in others 140 seeds are required. The period of maturity ranges from 90 to 135 days. Seventeen varieties of the fifty-one analyzed contained 40% protein. The difference in protein content ranged from 35% to 44.8%, while the average for the whole number was 39.2%. The oil or fat content varied from 14.1% to 20.4% with an average of 18%. Only four varieties produced more than 20% of oil.

In determining the varietal differences in the oil content of soy bean seed, Garner, Allard and Foubert (29) grew a number of varieties under uniform conditions, used the purest seed obtainable, and repeated the tests for several seasons. Their data show

that there are enormous varietal differences in soy beans both as to size of seed and as to oil content. Furthermore, it should be noted that the seasonal effects of the three years did not influence the several varieties alike with respect to either of these two characters. More extensive tests through a period of five years and with several additional varieties fully confirmed these results. It is clear, therefore, that in soy beans heredity is a very important factor, not only with respect to the size and the oil content of the seed but also as regards the extent to which these characters respond to change in environment.

Soy beans—iodine values. One of the important uses for soy bean oil is as a substitute for linseed oil in the manufacture of paint and varnish. Its value for this purpose depends upon the amount of unsaturated acid contained. It follows that in a study of the factors affecting the oil content, it becomes of interest to learn what effect, if any, such factors have upon the iodine number which is taken as an index of the degree of unsaturation.

Stark (113) found that wide variations may exist in the composition (protein and oil content) of the same variety when grown in different localities of Illinois. Such variations as were observed in this investigation were apparently not due to geographical position or climatic conditions, but may probably be attributed chiefly to differences in soil fertility and soil reaction. Of the four varieties there was a greater difference in iodine value within one variety than existed between the other three *e.g.*, Black Eyebrow averaged 123.2, Manchu 131.6, Midwest 131.0, and Wilson Five 136.3. The differences within the varieties were, respectively, 5.1, 5.0, 7.4 and 3.7.

Cotton—oil percentage. When different varieties of cotton were grown under uniform conditions, using the purest seed obtainable and repeating the tests several seasons, marked varietal differences in size of seed and other important characters were noted, but the percentage of oil remained remarkably constant when the environmental conditions were the same (29). Williams (120) obtained somewhat greater variations in oil content in a test with 21 varieties.

Grape seed—iodine values. Investigations on grape seed oils indicate that the variety of the vine has a much greater influence on the composition of the oil than the region in which it is grown

(14). Analysis of 19 samples of oil prepared in the laboratory by extraction with trichlorethane and hot filtration through charcoal, from seed of known origin, gave the following minimum, average, and maximum results, respectively: yield 7.1, 15.2, 20.0%; specific gravity at 15° C., 0.924, 0.0933, 0.950; viscosity at 35° (in dynes per sq. cm.) 0.2723, 0.3500, 0.4237; viscosity at 57°, 0.1342, 0.1800, 0.2423; acidity (as oleic acid) 0.24, 0.73, 1.80%; refractive index 1.4723, 1.4767, 1.4797; saponification number 190.5, 197.6, 208.0; iodine number (Hanus) 115.3, 126.4, 132.6; acid number (Andra) 12.8, 20.7, 29.3.

The number of carbon atoms in glycerides. It is significant that all fatty acids in fats contain an even number of carbon atoms, and that those of 18 carbon atoms (C_{18}) predominate, the carbon chain being a multiple of the carbohydrate unit six (C_6).

The three main fatty acids. While there is a difference in the relative amounts of different acids in different oils, there seems to be little difference in the nature of these acids, these usually being oleic, linoleic and linolenic.

In the case of oleic acid, for which a number of isomerides are possible with different positions of the double linking, the natural acid is practically always the Δ^9 acid, that is, it is the one with the double bond between the ninth and tenth carbon atoms, beginning with the carbon of the carboxyl (COOH) group. The existence of this acid is doubtless related to its activity, since the activity varies considerably with the position of the double linking in the carbon chain. This may show that while an organism may, in the course of evolution, acquire the ability to produce new substances, that which it had been producing in its primitive state it continues to produce in exactly the same form. The particular oleic acid found in plants must have been formed in the early stages of the evolution of the vegetable kingdom (103).

An examination of the relation between the value of the iodine number and the nature of the unsaturated acids contained in different vegetable oils shows that a low value of this number does not indicate the exact nature of these acids. In some cases the degree of unsaturation is due to a small amount of highly unsaturated acids, while in others it is due to a large amount of more nearly saturated acids. In most cases, the high unsaturation corresponded to the predominance of linoleic acid. This was proved by an examination

of the amount of crystalline tetrabromide and hexabromide obtained from the saponified oils (103).

ALIPHATIC ACIDS SPECIFIC TO FAMILIES

Seed fats from plants belonging to the same or closely allied families often contain the same fatty acids, and several families are characterized chemically in that one or more fatty acids predominate in the oils of all the species. The literature shows that such relationships have been recognized for a long time, and the names of the individual fatty acids indicate the plant families: Lauraceae—lauric acid; Myristicaceae—myristic acid; Linaceae—linoleic acid, linolenic acid. In a recent detailed analysis (38) of the mixed fatty acids of various seed fats from some of the Palmae, Cruciferae and Umbelliferae, together with earlier data on other members of these families and of the Myristicaceae, the fatty acids have been compared in detail. In any of these four families the composition of the fatty acids of the seed fats is of the same general type, but each family is marked by definite and specific characteristics in the composition of the fatty acids. Thus the Palmae seed fats almost always contain 46%–50% of combined lauric acid, with minor amounts of caprylic, capric, myristic and palmitic acids, and relatively small proportions of oleic acid; in the Myristicaceae seed fats myristic acid predominates; in cruciferous seeds there is usually 40%–50% of combined erucic acid, the remainder consisting of oleic and linoleic acids in varying proportions; and umbelliferous seeds appear to be characterized by an isomeric form of oleic acid, petroselinic acid (20%–75%), which has not yet been observed in the seed fats of any other family except the closely related Araliaceae. Both the Umbelliferae and the Araliaceae belong to the order Umbelliflorae. Other characteristic acids are the cyclic acid, chaulmoogric group, of the Flacourtiaceae (114). And then there are the “carriers” of specific color reactions, as the chromogen of the Halphen reaction in the oils of the Malvae. It is inferred that the seed fats of any botanical family have certain characteristics of their own and much in common with each other, differing from those of dissimilar families. This is not universal, however; in the Euphorbiaceae, for example, castor oil from seeds of *Ricinus communis* is entirely different from the oils of the genera *Aleurites* and *Mercurialis*. The predominance of one acid in the oils from a

family is confined only to the seed or kernel oils, and the oil or fat from other parts of the plant may be entirely different; *e.g.*, palm oil from the fruit pulp of the oil palm contains no lauric acid. Hil-ditch has devised a useful method for separating the glycerides in the oils, and therefore for determining the exact manner in which the fatty acids are grouped together in the glycerides. This work has shown that in the seed fats there is often a similarity, even between the actual glycerides present in closely related plants, and also that there tends to be an even distribution of the various fatty acids throughout the glycerides. Hence in the seed fats mixed glycerides are the rule, and simple glycerides occur only when one fatty acid predominates to such an extent as to render their formation unavoidable, *e.g.*, trimyristin in nutmeg fat. This, however, does not hold for the glycerides in parts of the plant other than the seed, *e.g.*, in fruit pulp or leaves; these resemble animal fats in that there is no tendency toward an even distribution of the fatty acids, and therefore simple glycerides are more common (39). Again, in seeds storing fat in both embryo and endosperm, the composition of the fat may be quite different in the two tissues.

INCREASE IN NUMBER OF C-ATOMS IN ACIDS AND NUMBER OF ACIDS
IN SEED FATS WITH ADVANCE IN EVOLUTIONARY POSITION

In the latest compilation of analyses of seed fats (39*a*), data from 16 natural orders (24) are given. When the component acids of the families of these orders was considered (88) it was found that seven orders had an increase in the number of acids, eight had an equal number of acids and one had a decrease in the number of acids with an advance in evolutionary position of their constituent families.

When the number of carbon atoms of these acids was considered (88) it was found that eight orders had an increase in the number of C-atoms, six had an equal number of C-atoms and two had a decrease in the number of C-atoms with an advance in evolutionary position. If, however, the terminal families of those analyzed of the Malvales, Myrtiflorae, Contortae and Tubiflorae (*i.e.*, respectively, Sterculiaceae, Myrtaceae, Asclepiadaceae and Acanthaceae) be removed from consideration, then three of these four orders showed an increase in the number of acids and all four showed an increase in the number of C-atoms in these acids with an increase

in evolution. An increase in the number of C-atoms indicated in these instances an increase in molecular weight of the acids which contain them.

RANGE AND AMPLITUDE OF IODINE VALUES IN SPECIES, GENERA AND FAMILIES

The properties of a vegetable oil of any plant in any part of the world can be foretold from two factors, its place in the botanical system and its climatic source. By determination of the iodine number different species of the same genus grown under similar climatic conditions showed a similar content of unsaturated fatty acids in the fatty oils derived from them (64). Experiments conducted to study the influence of climate on the composition of fatty oils showed that oils containing glycerides of unsaturated acids with one double bond (oleic, erucic or ricinic) are indifferent toward climatic changes, while in acids with three double bonds (α - and β -linolenic acid) the iodine number decreased with increase of temperature or with growth of the plant in more southerly regions. Oils with two double bonds (of the linoleic acid type) hold an intermediate position.

As a criterion, the iodine number may be used, since it is easy to determine and indicates changes of composition usually with sufficient clarity. For example, for linseed oils in the region of Moscow during the period of 1907–1916, Ivanov (58) found the iodine number to be 180 ± 4 ; for sunflower of Veron, the iodine number for a 25-year period averaged 125 ± 3 ; for the region about Cuba, in the case of the same oil, the number was 122 ± 3 . The relative constancy of the composition applies—for a constant ecological factor—not only for oils of the same botanical variety, but also—though to a rather limited extent—to fats of allied species of the same genus, as tabulated on the following page.

The iodine number of a species can be approximately foretold by a knowledge of the climate in which it lives and the genus to which it belongs. For instance, the place of *Prunus divaricata* (alytascha) is well established in the genus *Prunus*, and geographically it is found in the Caucasus at 1.6–1.8 km. above sea level. These data fix the properties of its oil; it must resemble almond oil, but on account of the higher altitude of its habitat its percentage of unsaturated acids must be greater. The actual results of analysis of

AVERAGE VARIATIONS OF THE IODINE NUMBERS OF OILS OF PLANTS OF THE SAME GENUS

Genus	Family	No. of species studied	Vicinity of cultivation	Iodine No. (Range of variation)
<i>Malva</i>	Malvaceae	5	Leningrad	124 ± 4
<i>Hibiscus</i>	"	4	Moscow	121 ± 5
<i>Gossypium</i>	"	3	Taschkent	108.7 ± 5
<i>Lavatera</i>	"	6	Turkestan & Cuba	120 ± 8
<i>Trollius</i>	Ranunculaceae	3	Moscow	139 ± 5
<i>Aconitum</i>	"	9	"	115 ± 7
<i>Delphinium</i>	"	6	"	112.3 ± 8
<i>Thalictrum</i>	"	7	"	174 ± 12
<i>Aquilegia</i>	"	5	"	191.4 ± 13

seven samples of oil from kernels of *alyschka* confirm this conclusion (64).

The maximum range in iodine numbers for the oils of families may also be established. For instance, the published analyses of the oils from members of the Anacardiaceae lead to the conclusion that this family yields oils containing principally the glycerides of oleic acid, and any linoleic glyceride that is present decreases in amount from the subtropic toward the tropic climate (64). The iodine number of members of the Anacardiaceae can not be below 25 nor above 120.

The oils in all members of most of the smaller families are in close agreement with each other, as is shown in the Cruciferae, Cucurbitaceae, Lauraceae, Myristicaceae, Pinaceae, Polygalaceae, Rutaceae, Sapindaceae, Sapotaceae, Simarubaceae, Solanaceae and Umbelliferae.

In the large families the oils of the different genera are usually in close agreement when grouped according to tribes. For instance (84):

Leguminosae¹

Subfamily I. Mimosoideae: (F) *Parkia*, (ND) *Pentaclethra*

Subfamily II. Caesalpinoideae: (SD) *Caesalpinia*

Subfamily III. Papilionatae:

Tribe 3, Genisteae: (SD) *Cytisus*, (SD) *Spartium*, (ND) *Lupinus*

Tribe 4, Trifolieae: (ND) *Trigonella*, (ND) *Trifolium*, (ND)

Ornithopus, (ND) *Melilotus*, (ND) *Medicago*

Tribe 5, Loteae: (ND) *Lotus*, (ND) *Anthyllis*

Tribe 6, Galegeae: (ND) *Galega*, (D) *Robinia*, (D) *Caragana*, (D)

Amorpha

Tribe 7, Hedysareae: (ND) *Arachis*, (ND) *Onobrychis*

- Tribe 8, Viciae: (SD) *Vicia*, (SD) *Cicer*, (SD) *Pisum*, (SD) *Lens*
- Tribe 9, Phaseoleae: (SD) *Voandzeia*, (SD) *Cajanus*, (SD) *Dolichos*, (SD) *Canavalia*, (SD) *Mucuna urens*, (SD) *Vigna*, (SD) *Phaseolus Mungo*, (SD) *Phaseolus lunatus*, (SD) *Phaseolus inamoenus*, (SD) *Phaseolus coccineus*, (SD) *Phaseolus vulgaris*, (D) *Glycine*
- Tribe 10, Dalbergiae: (F) *Dipteryx*, (F) *Pongamia*
- Gramineae
- Tribe 1, Paniceae: (D) *Panicum*
- Tribe 2, Maydeae: (SD) *Zea*
- Tribe 3, Oryzeae: (ND) *Oryza*
- Tribe 6, Andropogoneae: (SD) *Sorghum*
- Tribe 7, Phalarideae: (SD) *Phalaris*
- Tribe 12, Hordeae: (SD) *Secale*, (SD) *Triticum*, (SD) *Hordeum*
- Cucurbitaceae
- Tribe 1, Cumerineae: (F) *Hodgsonia*, (ND) *Telfairia*, (F) *Luffa*, (SD) *Acanthosicyus*, (SD) *Cucumis*, (SD) *Citrullus*, (SD) *Cucurbita*, (SD) *Bryonia*
- Tribe 3, Elateriae: (SD) *Echinocystis*
- Guttiferae
- Tribe 2, Moronobeae: (F) *Symphonia*, (F) *Pentadesma*
- Tribe 3, Garcinieae: (F) *Garcinia*
- Tribe 4, Calophylleae: (ND) *Calophyllum*, (ND) *Mesua*

ADVANCE IN PHYLOGENETIC POSITION IN THE CRYPTOGAMS AS INDICATED BY THEIR FATS

It has already been determined that seed fats of tropical angiosperm families have lower iodine values (85.3), lower molecular weights and higher melting points than the seed fats of temperate angiosperm families (124.0). It has likewise been shown that when seed fats of these families are first separated according to climate of habitat, the higher the plant family is in evolutionary position the more likely it is to form seed fats with large iodine numbers, higher molecular weights and lower melting points. There are also indications of an increase in the number of acids in seed fats with advance in evolutionary position (83, 86, 88, 89).

It is of interest, therefore, to find experimental evidence in favor of a similar situation in the lower plants. In Table II five organisms are listed in evolutionary sequence. From the figures it is apparent that the fats with the highest iodine values are produced at the lowest temperature, and in *Aspergillus niger* the fat produced at a temperature intermediate between the highest and the lowest has also an intermediate iodine value. The temperature and mean iodine values are, respectively: for I. Timothy grass bacillus 14°

¹ The abbreviations used are as follows: D, drying oil; SD, semi-drying oil; ND, non-drying oil; F, fat.

TABLE II

Plant	Temperature (degrees C.)	Reaction time (days)	Molecular weight (mean)	Iodine number of fat
I. Division, Schizophyta, Timothy grass bacillus	14° 35°	57-59 (116) 31-35 (116)
XI. Division, Eumycetes 1. Class, Phycormycetes <i>Rhizopus nigricans</i>	12° 25°	30 10 13	289 288 287	87.2-88.7 (mean 88) (101) 79 (101) 77.3 (mean 78) (101)
2. Class, Ascomycetes <i>Aspergillus fischeri</i> <i>Aspergillus niger</i>	20° 37° 18°	16 12 17 56 302 323-330	93 (104) 88 (104) 146.7-153.6 (101) 145.9-150.2 (mean 149) (101) 124 (101) 132.5 (101) 131.3 (101) 127 (101) (mean 129) (101) 92.1 (101) 92.3 (101) 99.9 (mean 95) (101)
4. Class Basidiomycetes <i>Sterigmatocystis nigra</i>	17° 35°	112-116 (116) 83-87 (116)

(iod. val. 58), 35° (33); for II. *Rhizopus nigricans* 12° (88), 25° (78); for III. *Aspergillus fischeri* 20° (93), 37° (88); for IV. *A. niger* 18° (149), 25° (129), 35° (95); and for V. *Sterigmatocystis nigra* 17° (114), 35° (85).

There are also indications that the fats with the highest iodine values and highest molecular weights are also produced by the plants highest in evolution when these plants are grown at comparative temperatures, *e.g.*, I. 14° (iod. val. 58), II. 12° (88) (mol. wt. 289), III. 20° (93), IV. 18° (149) (m.w. 323), and V. 17° (114) or I. 35° (33), II. 35° (78) (m.w. 287), III. 37° (88), IV. 35° (95) (m.w. 290) and V. 35° (85). Conversely, as shown by the above figures, there are indications that the fats with the lowest iodine values and lowest molecular weights are produced by the plants lowest in evolution when these plants are grown at comparative temperatures.

EVOLUTIONARY STATUS OF SPERMATOPHYTE FAMILIES IN RELATION TO THEIR FATS

Some 318 fats have been analyzed from 83 spermatophyte families. As there are 295 such families, about 30% of them have thus been analyzed (86). The families that produced these materials were divided into climatic groups as follows: tropical, tropical-subtropical, subtropical, subtropical-temperate, temperate and widely distributed. By far the most of these families were in the tropical, temperate, or widely distributed groups. The tropical and temperate families that contain fatty oils were tabulated in (86). These two zones, tropical and temperate, were chosen for discussion because they represented extremes in climatic difference.

The tabulated list of tropical fat families begins with the Palmae and Araceae and closes with the Rubiaceae and Cucurbitaceae, and includes many quite evenly dispersed families between these limits. For example, there were two families with botanical serial numbers below 1000, three between 1000 and 2000, eight between 2000 and 3000, four between 3000 and 4000, ten between 4000 and 5000, fifteen between 5000 and 6000, eight between 6000 and 7000, five between 7000 and 8000, and two between 8000 and 9000 (17). Consequently the group presented a representative cross section of all tropical families.

These dispersals of data were graphically portrayed in the scatter

diagram (Fig. 3 in 86) and showed that any additional data will fall within the scope of the statistics already obtained.

From these tabulated data it was definitely determined that there was a consistent variation in some of the chemical and physical properties of these materials according to the tropical and temperate climates in which they were produced. For instance, it was found that the tropical and subtropical fats had higher melting points and lower iodine values (*i.e.*, they were more saturated) than the fatty oils of temperate climates (83). This finding was supported by the evidence of Hilditch (38) who, in analyzing fat constituents, found specific acids for four plant families, namely lauric (mol. wt. 200, m.p. 48° C.) for the Palmae, myristic (mol. wt. 228, m.p. 58° C.) for the Myristicaceae, erucic (mol. wt. 338, m.p. 33.5° C.) for the Cruciferae, and petrosilinic (mol. wt. 282, m.p. 14° C.) for the Umbelliferae. As the Palmae and Myristicaceae are tropical and the Cruciferae and Umbelliferae temperate, the average molecular weight of the tropical families, 214, is lower than that of the temperate, 310. At the same time the average melting point of these tropical acids, 53°, is higher than that of the temperate, 23°.

NATURAL OR EVOLUTIONARY SYSTEMS OF PLANTS

Several systems of plant classification have been developed, such as those of Bentham and Hooker (8), Engler and Prantl (24), Bessey (10), Rendle (106), Hutchinson (43) and Mez (95). These systems vary in the arrangement of their families, not only as to the relative positions of the families within the systems, but also as to the families chosen for origin and termini. They all agree, however, in having more primitive families as origins and more highly organized families as termini. For convenience, Engler and Prantl's system was chosen to illustrate the relative general evolutionary position of the plant families in respect to the chemical compounds formed by them. In this paper the serial numbers given these families by DeDalla Torre and Harms (17) were used.

In giving serial numbers to the various plant families in the Engler and Prantl system, DeDalla Torre and Harms begin with the most primitive plants and continue to the most highly organized. The arrangement of plant families in accordance with their evolutionary positions assumes a tree-like form with the more primitive

families at the base of the tree and the most highly organized families at the tips of the branches, the topmost families on the tree representing the most highly evolved plants. It might appear at first glance that a linear system of numbering would not truly represent the proper relative positions of the various families in their order of evolution. Such, however, is not the case, as the following examples will show. Gymnosperms are treated first and have smaller numbers than angiosperms. In angiosperms the monocotyledons have lower numbers than the dicotyledons. In the dicotyledons the members of the subdivision of Archichlamydeae have lower numbers than have the Metachlamydeae. Of the Metachlamydeae the order Diapensiales is succeeded by the Ebenales, Contortae, Tubiflorae, Rubiales and Cucurbitales.

Each order has the most primitive family as its lowest number and the latest family has the highest number. In the DeDalla Torre and Harms numbering of the various Engler and Prantl genera it is as though the evolutionary tree were stripped of its branches and each branch, beginning with the lowest on the tree, were laid tip to base in a horizontal line which terminates with the highest branch.

In order for evolution to have taken place in a tree-like form, it is necessary that a lower branch be older (more primitive) than the one above it. Consequently each branch base represents a stage in progressive development which can be correctly expressed by serial numbers. Likewise the apical tips of the branches typify steps in the evolutionary progress of the plant families which also can generally be accurately shown with respect to each other by serial numbers. It may be, however, that the tip of a lower branch portrays a higher stage than the base of the branch next above it. If such is the case, it is nevertheless true that this higher stage is not higher than the base of the branch to which the tip belongs. Therefore it is shown that the average serial number of a lower branch must generally be lower than the average serial number of the branch just above it. Consequently, although the serial numbers of DeDalla Torre and Harms constitute a linear system, they are nevertheless sufficiently representative of the tree-like natural system of plant evolution for our purpose. The position of the orders on Figure 3 (in 86) showed this to be true. Should an individual family be wrongly placed in the system, use of the average botanical number of groups of families tends to eliminate such error.

EVOLUTIONARY STATUS OF FAMILIES IN RELATION TO THE
IODINE VALUES OF THEIR FATS

In table 2 (in 86) the temperate fatty oils were separated from those produced by tropical plant families. It is apparent from this table that temperate fatty oils have higher average iodine values (124.0) than tropical (85.3).

In Figure 3 (in 86) the data of the tropical fats from Table 2 (in 86) were arranged in a scatter diagram. Each point represented the average iodine number of the fats obtained from a certain family, which family was indicated by its numerical position in the Engler and Prantl system. A vertical line connecting various points showed the families of an order.

To determine the trend of these points, a straight line was used. A straight line was chosen because it constitutes the best means of showing a trend. There was only one straight line which fitted most accurately the plotted data. The constants of this line of best fit were determined by the method of least squares (96). It was assumed that the botanical numbers were free from any error; therefore, they were used as independent variables. The resulting equation was

$$y = 78.98834 + 0.00013287x$$

where x indicates the botanical numbers and y the average iodine numbers of the fats. This equation had a positive slope (+0.00013287) which showed the line to have an upward trend.

If both the botanical numbers and the molecular weight were considered as not free from error, the trend would have had a similar nature.

It can be definitely stated, therefore, that the higher the tropical plant family is in evolutionary development, the greater will be its tendency to produce fats of large average iodine numbers (and also the lower will be their melting points).

ONTOGENY VERSUS PHYLOGENY IN RELATION TO FAT FORMATION
AND PERCENTAGE

It is now a well known fact that in the formative development of seed, carbohydrate is changed to a highly saturated fatty oil and this fatty oil is in turn changed to a less saturated fatty oil. If chemical ontogeny is a recapitulation of chemical phylogeny then

one might apply this test to the monocotyledons and dicotyledons to determine which group originated first. Such a test might also be applied to the Archichlamydeae and Sympetalae.

The taxonomic distribution of oil and starch in seeds has already been tabulated (85). These data were reclassified (Table III) as to the percentages of starchy and oily embryos in the seeds of Monocotyledonae, Archichlamydeae and Sympetalae. The percentages of starchy and oily embryos in these groups were also determined as well as the nature of the total seed contents.

An examination of the data in Table IV showed a definite increase in the percentages of families with oily embryos, oily albumen and oily general contents in the series beginning with the monocotyledons, through the Archichlamydeae to the Sympetalae (91). There was a corresponding decrease in the starchy contents. The monocotyledons also had less saturated seed oils than those of the Archichlamydeae and Sympetalae (87). From these results it was deduced that the monocotyledons in their present chemical development are more primitive than the dicotyledons. Therefore the monocotyledons may have originated before the dicotyledons or as an early branch from the primitive dicotyledons. As a result of a similar deduction it is stated that the Archichlamydeae are more primitive chemically at least than the Sympetalae.

Use of Chemical Factors in the Study of Phylogeny. The iodine values of seed fats have been used in the comparison of plant families and orders as well as in the comparison of different plant groups, *e.g.*, the monocotyledons with the dicotyledons. It has already been shown (86), first, that the more closely plants are related, the more similar are their chemical products; and second that the more highly evolved the plant (according to the Engler and Gilg (23) classification) the larger are the iodine values of its fats, provided the plants grow in the same climate. These findings have been made use of in further comparisons of angiosperm phylogeny.

The arrangement of families and orders in the Bessey system is to be found in Bessey (9), and the arrangement of families and orders in the Engler and Gilg system was taken from Engler and Gilg (23).

In the discussion of results obtained in the tables, the first column (in Tables III to VI inclusive in 87) includes all the tropical families from which data have been obtained, while the other columns

TABLE III
PERCENTAGES OF FAMILIES THAT HAVE STARCHY OR OILY EMBRYOS, ALBUMEN, AND GENERAL CONTENTS

Plant group	Embryos		Albumen		General contents oily %
	Starchy %	Oily %	Starchy %	Oily %	
Monocotyledons	4/33 = 12	15/33 = 45	19/33 = 57	9/33 = 27	5/33 = 15
Archichlamydeae	21/137 = 15	62/137 = 45	23/137 = 16	43/137 = 30	69/137 = 50
Sympetalae	4/42 = 9	18/42 = 42	1/42 = 2	17/42 = 40	23/42 = 54

include only part of these families. Therefore the left hand column gives a better average value founded on a larger group of plant families than any of the other columns. The figures in it can consequently be considered as more representative and dependable than those in the other columns.

Are the Magnoliaceae pre-Ranunculaceae? Hallier (36, 37) believed that the Ranunculaceae and Nymphaeaceae were descended from the Magnoliaceae through the Schizandraceae, Lardizabalaceae and Berberidaceae. Engler, however, has concluded that it is not likely that such characteristically woody families as the Magnoliaceae and Lauraceae have arisen from herbaceous ancestors, or *vice versa*, but that these woody types have had a quite different origin from the herbaceous Ranunculaceae, as have most of the monocotyledons whose protangiospermous ancestors may be assumed to have been herbaceous (12).

Fats have been found in the Ranunculaceae, Berberidaceae, Lardizabalaceae. All three of these families are found for the most part in the temperate zone, and consequently they can be successfully compared chemically. Table I (in 87) shows that the iodine values of the fats diminish from the Ranunculaceae to the Lardizabalaceae. Consequently, Hallier's theory of descent has chemical support as far as chemical data are available.

The Ranunculaceae consist mainly of herbs, the Berberidaceae of herbs and shrubs, and the Lardizabalaceae of shrubs. Here there is an indication that shrubs may precede herbs phylogenetically.

The Magnoliaceae are mainly a sub-tropical family and therefore can not be compared chemically with the three temperate zone families considered here. The average iodine number of the fats of the Magnoliaceae, 95.5, should be lower than the temperate fats and higher than the tropical fats in order to comply with the general rule. It was assumed that the Magnoliaceae are plants of the temperate zone as are the three other families; their low value, 95.5, therefore would indicate that they are more primitive than the Berberidaceae and Ranunculaceae.

Are Herbs Derived from Trees? The foregoing chemical results indicate that herbs may be derived from trees. Eames (21), in a paper devoted to the subject, brought forward evidence that the earliest dicotyledons possessed a solid tubular woody cylinder of considerable thickness which has gradually been reduced and finally

broken up into a circle of separate strands, characteristic of the "typical" herbaceous condition. Such an hypothesis of reduction from primitive arborescent forms has also been worked out under the direction of E. C. Jeffrey by several other members of his laboratory (2, 4, 70). In more recent papers (109, 110) there is evidence in support of this view from palaeobotany, phylogeny, anatomy and geographical distribution. It is no wonder, then, that Bessey (10) included in his "General principles adopted for the classification of plants" the postulate that "in certain groups, trees and shrubs are probably more primitive than herbs".

This hypothesis has been considered from the standpoint of the chemical products derived from plants. In Table II (in 87) the fats from tropical plant families were considered in this respect.

From the final average obtained of the iodine values of fats, there is a clear indication that trees produce fats of lower iodine values than shrubs, and that shrub fats have lower iodine values than those of herbs. There is chemical support, therefore, for the contention of Bessey (10), Sinnott and Bailey (109), and others, that angiosperm herbs have been derived from woody plants.

Do Dicotyledons Precede Monocotyledons? In the history of the development of taxonomic systems, there is a difference of opinion among authors as to the precedence of monocotyledons or dicotyledons in the natural system. John Ray (105), de Jussieu (74), Eichler (22), Engler and Prantl (24), Rendle (106) and Johnson (72) considered monocotyledons as the antecedents of dicotyledons. The following botanists have believed dicotyledons to be the forbears of monocotyledons: de Candolle (13), Bentham and Hooker (8), Wettstein (119), Bessey (10), Hallier (36, 37), Clements (15), Jeffrey (71), Hutchinson (43).

Bessey's (10) phylogenetic postulate is that dicotyledons are more primitive in origin than monocotyledons. Chemical results indicate, however, that monocotyledons preceded dicotyledons or may have been an early branch from the dicotyledons or may have had a separate origin. In Table III (in 87) the iodine values of fats are higher in dicotyledons than in monocotyledons, being 85.85, 109.40 and 77.92 in the dicotyledons, and 63.62, 80.98 and 28.90 in the monocotyledons.

Families in the monocotyledons and dicotyledons were given according to the Engler and Prantl system. The chemical data used

were from McNair (86). The families of the monocotyledons and dicotyledons were grouped according to the predominance in them of herbs, shrubs and trees; herbs; herbs and shrubs; shrubs and trees; and trees.

Polypetaly versus Gamopetaly. In his consideration of the flower types, Bessey (10) considers that "free petals (polypetaly) are more primitive than connate petals (gamopetaly)". The Bessey system divides both the Axiflorae and Calyciflorae into Apopetalae (Polypetalae) and Gamopetalae. But in the Engler and Prantl classification the Apopetalae are found in the Archichlamydeae and the Gamopetalae in the Metachlamydeae.

This postulate of Bessey has found support in the chemical products of the Polypetalae and Gamopetalae. For instance, the fats (Table IV in 87) 82.57 vs. 84.40 and 95.19 vs. 110.79. In Engler and Prantl's system the Archichlamydeae (Apopetalae) are according to both phylogeny and chemical products more primitive than the Metachlamydeae (Gamopetalae). This was shown by the fat iodine values (Table IV in 87) 82.94 vs. 93.84, 76.77 vs. 86.82.

Polycarpy versus Oligocarpy. Bessey (10) advanced another postulate which has found chemical evidence in its favor. This postulate is that "many carpels (polycarpy) preceded few carpels (oligocarpy)".

Bessey separates the Polycarpellatae from the Dicarpellatae in his Gamopetalae. Chemical evidence for these divisions in the Bessey system was found in the fats (Table V in 87). However, the fats do not support the Bessey theory as shown by Table V: 88.04 vs. 83.74 and 88.04 vs. 59.20.

Apocarpy versus Syncarpy. In his consideration of the evolution of flower structure, Bessey (10) states: "free carpels (apocarpy) are more primitive and from them connate carpels resulted; sometimes, however, when the carpels have remained loosely united during the evolution, they may again become quite free".

In Bessey's classification the Apopetalae have separate or united carpels, but the Gamopetalae have united carpels only. Consequently, as was shown above under "Polypetaly versus Gamopetaly", chemical evidence indicates that free carpels are more primitive (Table VI in 87).

The Engler and Gilg system makes a clear separation of apocarpy from syncarpy in the Ranales (apocarpy) and Rhoeadales (syn-

carpy). The iodine values of the fats give favorable evidence to the theory, *e.g.*, (Table VI in 87) 72.03 vs. 105.95 and 72.86 vs. 92.40.

Engler and Gilg System from a Chemical Standpoint. The various divisions under the Engler and Gilg system of classification were divided for the purpose of this study into the following various sections such as the monocotyledons: A, the Amentiferae (from the Piperales to Urticales); B, Proteales to Polygonales; C, Centrospermae; D, Ranales to Umbelliflorae; A, Diapensiales to Plumbaginales; B, Ebenales; C, Contortae; and D, Tubiflorae to Campanulatae. These divisions or sections were considered as radii originating from a central point, thus forming a cart wheel design. This central point was taken as zero, and the positions of the various orders on the radii were taken as equal to the chemical results obtained from a study of the iodine values of their fats. If one looked down upon a tree from its top the branches would appear to radiate from a common center. In a similar way the branches of the phylogenetic tree were presented.

The iodine values of the fats (Fig. 1 in 87) of tropical families and sections of the Engler and Gilg system show, in general, a close agreement with the botanical classification. Complete agreement is found in the Monocotyledonae, Amentiferae and Metachlamydeae.

According to the Engler and Gilg phylogenetic system, the Santalales are farther advanced than the Proteales. The iodine values, however, indicated the reverse to be true. Inasmuch as the chemical differences between them was very slight (Proteales 85 and Santalales 83) the chemical evidence was hardly sufficient to counteract the morphological evidence and phylogenetic position.

Likewise the chemical evidence presented is probably too insignificant to warrant serious consideration for departure in phylogenetic position from Engler and Gilg classification, namely, that the Parietales 70.57 should phylogenetically precede the Ranales 72.03, or that the Geraniales 65.82 should precede the Ranales 72.03, or that the Sapindales 79.24 should precede the Malvales 90.65, or that the Umbelliflorae 91.8 should precede the Myrtiflorae 100.08.

The Bessey System from a Chemical Standpoint. The Bessey system (9), like that of Engler and Gilg, recognizes the two main

divisions of monocotyledons and dicotyledons. The dicotyledons, however, are divided differently than in the Engler and Gilg system. Bessey divides them into Axiflorae and Calyciflorae. In the Axiflorae, "axis flowers", the axis of the flower is normally cylindrical, spherical, hemispherical or flattened, bearing on its surface the hypogynous perianth, stamens and carpels (or the stamens may be attached to the corolla). In the Calyciflorae, "cup flowers", the axis of the flower is normally expanded into a disk or cup, bearing on its margin the perianth and stamens (or the latter may be attached to the corolla). The Axiflorae are considered as more primitive.

The groups of the Bessey system were considered as radii originating from a central point. This central point was taken as zero and the positions of the various orders on the radii as the various chemical results obtained from a study of their fats.

The iodine values of the fats (Fig. 2 in 87) of the tropical families and sections of the Bessey system showed in general a close agreement with the botanical classification. Only three of the orders did not agree with the botanical classification, *viz.*, the Caryophyllales, Celastrales and Ranales. The Caryophyllales 105 should precede the Ebenales 87. The Celastrales 110 should precede the Umbellales 92. The Ranales 80 should be nearer the point of origin than any of the other dicotyledons.

DISCUSSION OF THE ENGLER AND GILG AND BESSEY SYSTEMS FROM A CHEMICAL POINT OF VIEW

The Engler and Gilg system considers that monocotyledons precede dicotyledons. The Bessey system on the contrary classes dicotyledons before monocotyledons. The chemical evidence in regard to the iodine values of the fats (as given in 87) supports the Engler and Gilg contention: that monocotyledons may have preceded dicotyledons (Table III in 87).

With this exception both systems agree in their use of the following phylogenetic principles:

- a) Polypetalous flowers are more primitive than gamopetalous flowers.
- b) Numerous carpels represent a more primitive condition than few carpels.
- c) Separate carpels represent a more primitive condition than united carpels.

All of these, except *b*, have been shown to hold true from the chemical investigations. In this case chemical evidence (Table V in 87) indicated that few carpels may represent a more primitive condition than many carpels. The Bessey, and the Engler and Gilg systems, however, differ in the relative importance given to these phylogenetic principles.

In both systems the Ranales did not fall in the position allotted to them. There was, however, better agreement with their position in the Engler and Gilg system than with their position in the Bessey system.

The Caryophyllales and Gentianales are far removed in the Engler and Gilg but may agree better with that classification than with Bessey's (Fig. 1 in 87).

The Celastrales and Umbellales did not harmonize with the Bessey system (Fig. 2 in 87). These were in better accord in Engler and Gilg.

SUMMARY

The oil content of seed varies with the successive stages of maturity. During ripening there is an increase in the amount of unsaturated acids of drying oils, whereas the composition of non-drying oils may remain practically the same.

Maximum oil production may be reached and further production cease when chlorophyll disappears. However, increase in the iodine value may continue after disappearance of the chlorophyll.

Saturated fatty acids are formed first but are progressively transformed into acids with a greater degree of unsaturation.

Considering only percentage of oil, there is a very sharp increase during the first few weeks after blossoming in soy beans, and then only a slow gain until near the end of ripening. During the final stage of ripening there is a decrease in both the size of the seed and in oil content.

The iodine number increases quickly at the last stage of maturity, there being little difference in the early stages.

In sunflower oil, the iodine value was found to remain almost constant during ripening, but the saturated acids decreased steadily in amount while the unsaturated acids increased in amount.

In ripening seeds increase in the percentage of oil is accompanied by a decrease in the carbohydrate percentage.

The total percentage and iodine value of oil is affected more by the character than the length of the growing season.

Vigorously vegetative plants have lower concentrations of fat in their leaves than weakly vegetative plants.

Different varieties are not always influenced in the same manner by environment. Percentages of oil produced are influenced by both heredity and environment.

The comparative effect of soil types on per cent of oil formation varies with seasonal conditions. In some seasons clay soil produces a higher percentage of oil than loam, and *vice versa*.

Under practical conditions climate is a more potent factor than soil in modifying the oil content of seed.

Bacteria have been found to produce the most fat when in neutral media and the least when the media are acid or alkaline.

Increased amounts of fat are formed by mold when grown in neutral or slightly alkaline media, a high concentration of glucose, a low concentration of ammonium nitrate and at high temperatures.

Within the limits ordinarily met in farm practice the relative fertility of the soil does not greatly influence size of the (soy bean) seeds or oil content. Increased applications of nitrogen lowered considerably the oil content of the kernels. Increased applications of phosphorus and potassium had no appreciable effect.

Additions of phosphorus to pot cultures of soy beans resulted in increased oil content, but addition of potassium alone gave practically no increase in oil content.

In seedling cultures of *Picea*, potassium-free cultures gave stronger reactions for fats in the root systems. When phosphorus was omitted fats were absent.

Linseed oil had a higher iodine value when produced on non-fertilized fields.

More saturated fats are produced by fungi at lower temperatures.

Lower saturated acids are first formed and higher unsaturated acids are formed later.

Mild warm climate favors formation of saturated fatty acids and of unsaturated acids with one double bond. Cold climate favors formation of unsaturated acids with two or three double bonds.

Starch in evergreen leaves (*Linnaea*) forms at moderately elevated temperatures and is reconverted into oil when the temperature is gradually lowered.

As plants grow nearer to the equator the calorific value and the iodine value of their fats decrease. This also occurs in transplanted plants.

Oil from the heartwood of deodar has an increased iodine value when the trees are grown at a higher altitude. This is also true of the fats of the seeds of many plants.

Fats in British brown algae (*Phaeophyceae*) show an increase in amount and in iodine value with an increase in depth of immersion.

Shade increased the starch content and decreased the oil content of flax seed. Heavy shade lowered the iodine value.

Increased moisture content of the soil has been found to increase the total oil in flax seeds. The iodine value is likewise lowered by deficient soil moisture.

In seed germination absorption of water is accompanied by diminution in the amount of oil in the seed.

As in many higher plants, so in yeast: the equilibrium carbohydrate \rightleftharpoons fat is controlled by variations in water content.

Dense sowing of flax was found to increase the iodine value of oil.

In corn kernels crude fat segregates as a definite and distinct unit character and is inherited in accordance with Mendelian principles.

There are varietal differences in soy beans as to per cent of oil content as well as in iodine value.

In cotton the percentage of oil is hereditary but is greatly influenced by change in environment.

In grape seed oil the variety of the vine has a much greater influence on the composition of the oil than the region in which it is grown. This is shown in per cent and iodine value.

All fatty acids in fats contain an even number of carbon atoms.

Seed fats from plants belonging to the same or closely allied families often contain the same fatty acids, and several families are characterized chemically in that one or more fatty acids predominate in the oils of all species.

When the component acids of the families of 16 natural orders are considered, it is found that seven orders have an increase in the number of acids, eight have an equal number of acids and one has a decrease in the number of acids with an advance in evolutionary position of their constituent families.

When the number of carbon atoms of these acids is considered it is found that eight orders have an increase in the number of C-atoms, six have an equal number of C-atoms, and two have a decrease in the number of C-atoms with an advance in evolutionary position.

The properties of a seed oil of any plant in any part of the world can be foretold from two factors, its place in the botanical system and its climatic source. Different species of the same genus grown under similar climatic conditions show a similar content of unsaturated fatty acids.

The maximum range and amplitude in iodine values for the oils of families, genera and species may also be established. There is an increase in both range and amplitude with increase in size of the taxonomic group.

The oils of most of the smaller families are in close intrafamilial agreement, while those of larger families are often in better agreement if considered in tribal groups.

There is close agreement between the oils of species of a genus.

From the study of iodine values of cryptogam fats it has been found that fats with highest iodine values are produced at the lowest temperature, and that fats with the highest iodine values and highest molecular weights are produced by plants highest in evolution when grown at comparable temperatures, and *vice versa*.

From a study of iodine values of seed fats of spermatophyte families it can be definitely stated that the higher the tropical plant family is in evolutionary development the greater will be its tendency to produce fats of large iodine values (and also the lower will be their melting points).

In the formative development of seed, carbohydrate is changed to a highly saturated fatty oil and this fatty oil is in turn changed to a less saturated fatty oil. If chemical ontogeny is a recapitulation of chemical phylogeny, then applying the above chemical process, monocotyledons in their present chemical development are more primitive than dicotyledons, and the Archichlamydeae are more primitive chemically at least than the Sympetaleae.

By applying the two facts (*a*) that the more closely plants are related, the more closely similar are their chemical products, and (*b*) that the more highly evolved the plants the larger are the iodine values of their seed fats provided the plants grow in the same climate, the following phylogenetic deductions have been made:

The Magnoliaceae are more primitive than the Berberidaceae and Ranunculaceae.

Angiosperm herbs may have been derived from trees.

Monocotyledons are more primitive than dicotyledons.

Free petals (polypetaly) are more primitive than connate petals (gamopetaly).

Few carpels (oligocarpy) may have preceded many carpels (polycarpy).

Free carpels (apocarpy) are more primitive than connate carpels.

In a chemical comparison of Engler and Gilg's classification with Bessey's it is apparent that in both systems the Ranales do not fall in the position allotted to them but are in better agreement with their position in the Engler and Gilg system.

The Ebenales and Gentianales are out of harmony in the Bessey system, but in better agreement in the Engler and Gilg system (both in Contortae).

The Caryophyllales and Gentianales of Bessey are far removed in Engler and Gilg (being, respectively, Myrtiflorae and Contortae), and the chemical findings may agree better with that classification than with Bessey's.

The Celastrales and Umbellales do not harmonize with the Bessey system. These are in better accord with Engler and Gilg where the orders are considered as Sapindales and Umbelliflorae.

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GENE SEGREGATION IN AUTOTETRAPLOIDS

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The genetics of autotetraploids is complicated by various factors which are not encountered in the genetics of diploids. The most important of these complicating factors lies in the fact that one of the fundamental concepts underlying diploid genetics, that of "purity of gametes", is meaningless in the discussion of tetraploid genetics. In a tetraploid the gametes may be hybrid as well as "pure". This leads to a further complication in the zygotes, namely, that instead of having only one type of heterozygote, as in the diploid, we have three, which along with the two homozygotes gives five possible genotypes involving any one pair of genetic factors. These five genotypes are known as quadriplex, triplex, duplex, simplex, and nulliplex, according to whether there are four, three, two, one, or no dominant genes present.

Another complicating factor in the study of tetraploid segregation is the relation between certain cytological phenomena and the genetic ratios obtained. Diploid ratios involving a single gene are affected little or not at all by such variable factors as pairing, multivalent formation, non-disjunction, chiasmata, and the position of the gene on the chromosome in relation to the centromere, yet all of these factors have a profound influence upon tetraploid ratios. These points will be discussed in more detail later, but it should be pointed out here that while it is well recognized that variations in cytological behavior introduce variables in tetraploid segregation which make a precise mathematical analysis of the ratios difficult or even impossible, still it is felt that certain advantages are to be gained in a study of such ratios. The mere fact that tetraploid ratios are affected by cytological behavior opens up the possibility of studying the relationships between certain cytological and genetic phenomena, which cannot be studied in diploids where genetic ratios are, in general, more stable.

The literature on the segregation of genetic characters in autotetraploids falls into two main categories, those papers dealing with the theoretical aspects of the problem and those in which actual experimental data are presented and interpreted. While some of

the published work deals with both aspects, it has seemed most convenient to discuss these two categories separately, first treating the development of the theories concerning autotetraploid segregation, then reviewing the published data in the light of these theories.

REVIEW OF LITERATURE

Theories of Autotetraploid Segregation

The first report on segregation in autotetraploids appeared when Gregory (37) presented data on the tetraploid *Primula sinensis*. He attempted to explain his results on the basis of duplicate-factor ratios commonly found in diploids. Thus he assumed that the ratios he obtained on selfing a tetraploid hybrid corresponded to the 15:1 ratios found in diploids, and that the backcross ratios were 3:1. His conclusions were based on the false assumption that pairing must always take place between a chromosome derived from the maternal parent and one from the paternal parent (allosyndesis). Muller (61) pointed out this fallacy and assumed that pairing took place between any two of the four homologous chromosomes at random, independently of their origin. On this basis he concluded that the ratio obtained from selfing a duplex heterozygote should be 35:1, and that backcrossing such a hybrid to a homozygous recessive tetraploid should give a 5:1 ratio. Moreover, he attempted to show that Gregory's data fit these ratios better than those which Gregory himself had assumed.

No further theoretical treatment of the problem appeared until Haldane (38) published generalized formulae covering all types of polyploids. For autotetraploids he showed that, if one assumed random assortment of chromatids rather than of chromosomes, a duplex hybrid would give a ratio of 187:9 (or approximately 21:1) on selfing, and 11:3 on backcrossing to a homozygous recessive. These ratios have attracted considerable attention by subsequent authors, and data which seem to fit them are usually referred to as displaying "chromatid segregation". Along with this term, the term "chromosome segregation" has come into general use, referring to ratios which seem to fit more closely those given earlier by Muller. The unsoundness of considering these two types of segregation as separate and distinct fixed ratios, and of assigning observed data to one or the other, was brought out by Mather (59). However, the

practice has persisted to a certain extent to the present time. This point will be discussed later in more detail.

Mather published three papers (57, 58, 59) dealing in part with segregation in autotetraploids. These papers discussed the subject in the light of cytological facts, many of which were not well understood at the time the previous papers were published. In referring to the terms "random chromatid segregation" and "random chromosome segregation", he states: "It has been recognized by all later workers that these segregation . . . expectancies are in the nature of limiting types, and that the true segregations to be expected from autotetraploid organisms will actually lie somewhere between them. . .".

Further on in the same paper he makes this somewhat contradictory but probably sounder statement: "A new segregation was established (57) for the case of completely equational separation, and random chromatid segregation was shown to be the result of a combination of the two types in the random proportions of $1/7$ reductional and $6/7$ equational separation".

Thus the first statement refers to random chromatid segregation as a limiting type, while the second statement represents it as an intermediate type. This brings out an interesting contrast between the phenomenon of linkage of genes in diploids and linkage with the centromere in tetraploids, which has never been emphasized. In diploids the upper limit of crossing-over between two linked genes is 50%, and this limit is the equivalent of independent assortment or random segregation. In tetraploids, the upper limit of crossing-over between a gene and the centromere is likewise 50%, but this is not equivalent to random chromatid segregation. Instead, the ratios calculated on the basis of random chromatid segregation are equivalent to those which would be obtained if the amount of crossing-over between the gene and the centromere were approximately 43%.

One of Mather's most important contributions was the formulation of methods of calculating indices which could be used to characterize an observed ratio in terms of cytological behavior. These indices were based on the products of the amount of genetical non-disjunction in a quadrivalent (a), times the amount of equational separation of chromatids (e). The latter variable is in turn dependent upon the frequency of chiasmata, and hence the per cent

of crossing-over between the centromere and the gene in question. It was at first maintained (59) that the values for these variables would be the same in triplex and simplex hybrids, but would be different in duplex hybrids, where change of pairing partners might alter their value. Therefore the index calculated from simplex and triplex data was designated as alpha, while the one calculated from duplex data was called beta. However, this view has recently

TABLE I
AUTOTETRAPLOID RATIOS EXPECTED ON THE BASIS OF VARIOUS HYPOTHESES

<i>Mating</i>	<i>Gregory</i>	<i>Muller</i>	<i>Haldane</i>	<i>Mather</i>	<i>Mather's ratios when alpha is 1/3</i>
AAAA selfed	inf. 0	inf. 0	783 1	$64 - a^2$ a^2	575 1
AAAA \times AAaa	inf. 0	inf. 0	389 3	$48 - a - a^2$ $a + a^2$	107 1
AAAA \times Aaaa	inf. 0	inf. 0	769 15	$64 - 4a - a^2$ $4a + a^2$	563 13
AAAA \times aaaa	inf. 0	inf. 0	27 1	$8 - a$ a	23 1
AAaa selfed	15 1	35 1	187* 9	$35 - 2a - a^2$ $1 + 2a + a^2$	77 4
AAaa \times Aaaa	7 1	11 1	347 45	$44 - 5a - a^2$ $4 + 5a + a^2$	95 13
AAaa \times aaaa	3 1	5 1	11 3	$5 - a$ $1 + a$	7 2
Aaaa selfed	3 1	3 1	559 225	$48 - 8a - a^2$ $16 + 8a + a^2$	407 169
Aaaa \times aaaa	1 1	1 1	13 15	$4 - a$ $4 + a$	11 13

* Frequently called a 21:1 ratio.

been corrected (35), and it is now considered that one index will suffice to specify the segregations from all three types of tetraploid heterozygotes. The lower limit of this index should be zero, when there is no crossing-over between the gene and the centromere, or when no quadrivalents are formed. The upper limit should be 1/3 when there is 50% crossing-over between the gene and the centromere and random disjunction of quadrivalents.

The expected ratios based on the four hypotheses of Gregory, Muller, Haldane, and Mather are summarized in Table I, the last column giving the ratios expected on the basis of Mather's hypoth-

esis when alpha has its maximum value of $1/3$. It will be observed that there are nine types of crosses in tetraploids capable of giving segregating progenies, in contrast to diploids where there are only two such crosses, namely, the heterozygote times itself and the heterozygote times the homozygous recessive.

Experimental Data

Primula. The first experimental data on tetraploid segregation were published by Gregory (37), reporting on work with the tetraploid form of *Primula sinensis*. One of the pairs of factors upon which he reported was the *Thrum* (short-style) versus *Pin* (long-style) pair, designated by him as $A:a$ but referred to in the later literature as $S:s$. Data were presented involving four different mating combinations, namely, duplex and simplex plants selfed and each of these backcrossed to the nulliplex. When the data are compared with the expectancies based on his own hypothesis and the hypotheses of Muller and Haldane, the P values obtained are 0.27, 0.21 and 0.08, respectively. Due to the small populations involved, therefore, the data did not differ significantly from any of these three theoretical ratios. The other pair of factors studied was green versus red stigmas. It can also be shown that the data on this character did not deviate significantly from any of the three theoretical ratios. Thus, much of the discussion as to which ratios these data represented was statistically meaningless.

Further data on *Primula sinensis* appeared in an extensive report by Sömmе (77) who reported on the inheritance of seven pairs of factors, including the two discussed by Gregory. The conclusion was reached that none of the ratios obtained differed significantly from those postulated by Muller.

De Winton and Haldane (23) published results on three pairs of factors in *Primula*, including the S and G factors discussed by the two earlier authors and the $B:b$ factor pair for magenta versus red flower color. Particular attention was paid to linkage among these factors, since it had been shown in diploids that all three are located on the same chromosome. It was concluded that all three of these factors were located near the centromere, so that double reduction was disregarded. While there was a rather consistent excess of recessives in their data (as would be expected with double reduction), they attempted to explain part of these excesses by assuming

that some of the parents were pentasomic for the chromosome involved. However, in a later report on linkage in diploids (24), the data from tetraploid segregation were used to locate the position of the centromere on the linkage map of this chromosome. They stated that the data indicated that the centromere was close to the loci of the *S* and *B* factors but further distant from the *G* locus.

Datura. Blakeslee, Belling, and Farnham (9) published a report on the segregation of two factor pairs in tetraploid *Datura*. The value of these data, which lies in the extremely large populations involved, has apparently never been fully appreciated. Since no one has ever published a statistical analysis of the data, a comparison is included here between the observed ratios and the expectancies according to the Muller hypothesis (which the authors accepted), the Haldane random chromatid ratios, and the Mather ratios obtained by calculating alpha. In the case of the *purple:white* factor pair, a chi-square test gives a P value of only 0.012 when applied to the Muller hypothesis and less than 0.01 when applied to the Haldane ratios. On the other hand, when expectancies are calculated on the basis of Mather's alpha and compared with the observed data, a P value of 0.475 is obtained. Similar results are obtained from data on the *armed:inermis* factor pair, where both the Muller and Haldane ratios give P values of less than 0.01 while the Mather ratios give a P value of 0.15. Data from crosses involving triplex plants are not included in these chi-square tests, since the number of recessives expected from such crosses is too small. However, it should be noted that a total of 14 recessives appeared in populations where none was expected according to the Muller hypothesis.

Potato. In recent years the evidence indicating that the cultivated potato is an autotetraploid has created considerable interest in the genetics of this plant. The cytological evidence is conflicting in regard to the nature of polyploidy in the potato (13, 36, 29, 60). While the earlier workers were inclined to class this plant as an allotetraploid, some of the more recent writers, especially Cadman (13), favor the view that it is an autotetraploid. Meurman and Rancken (60) consider that it is fundamentally autotetraploid but have observed that there are only two instead of four chromosomes with satellites, indicating that some chromosome differentiation has taken place, so that the potato now has some of the characteristics of allotetraploids.

The genetic evidence as to the nature of polyploidy is likewise conflicting. All reports on the genetics of potatoes up to 1930 interpreted the inheritance as disomic. Müller (62) was the first to explain data on a tetrasomic basis, in connection with studies on resistance to dry rot. All of Lunden's and Jørstad and Lunden's earlier works (40, 53, 54, 56) interpreted the inheritance of genetic factors on a disomic basis, but in a more extensive study (55), based on very large populations, Lunden concluded that the segregation of all factors studied was tetrasomic. Some of these data fit the Muller ratios very closely, but in several cases there was an excess of recessives due to double reduction, and one factor gave segregations which were shown to fit the Haldane random chromatid ratios better than the Muller ratios. Since Mather's index of double reduction had not yet been published, no attempt was made to characterize the segregations on this basis.

Since Lunden's work there has been a tendency among most investigators to explain their results on a tetrasomic rather than a disomic basis. Krantz and his co-workers have explained the inheritance of pollen sterility on an autotetraploid basis (41). In their studies on resistance to common scab (42) they showed that the plants fell into five classes as regards breeding behavior, corresponding to the five possible genotypes in an autotetraploid. Stevenson, Schultz, and Clark (81) gave a tetrasomic explanation for the inheritance of immunity to Virus X. Cadman (12) has thoroughly reviewed the subject of autotetraploid inheritance in the potato and presented further evidence based on segregation of differences in reaction to virus inoculation. He has been the only worker up to the present time to employ Mather's formulae for obtaining an index of double reduction in potatoes.

The early papers by Black (5, 6, 7) all gave a disomic interpretation to the data on inheritance, but in his most recent paper (8) he has come to the conclusion that the potato is an octoploid rather than an allo- or autotetraploid.

The exact nature of polyploidy in the potato is a problem that is still far from being solved, by either cytological or genetic investigations. It is doubtless complicated by the fact that this species is a very old polyploid which has undergone considerable change since it first arose. It is highly probable that some of the chromosomes have become differentiated into two pairs so that their be-

havior is like that of an allotetraploid, while others have undergone little or no differentiation and continue to pair among themselves at random like the four homologous chromosomes of an autotetraploid.

Tomato. The first artificially induced tetraploid to be studied genetically was in the tomato. Sansome (73) investigated the inheritance of seven characters and concluded that three of them displayed "chromatid segregation", one displayed "chromosome segregation", one was intermediate, and in the other two the populations were not large enough to decide. As a matter of fact, the data indicate that with probably all of the factors, segregation was intermediate, as should be the case. When Mather later published his papers giving methods for calculating indices of double reduction, he drew on some unpublished data of Sansome for use in illustrative examples. In a cytological study of tetraploid tomatoes, Upcott (84) found that the amount of quadrivalent formation was clearly compatible with Sansome's genetic results.

Lindstrom (50) made a new approach to the study of quantitative genes of tomato by comparing their genetic behavior in tetraploids and diploids.

Dahlia. Cytological and genetic studies on the garden dahlia (43, 44, 45) have led Lawrence to conclude that *Dahlia variabilis* is an allo-octoploid resulting from doubling the chromosome number in a hybrid between two tetraploid species. At first he believed that some of the genetic factors displayed tetrasomic inheritance, while with others the inheritance was disomic. He assumed that the *Y* factor for flavone formation was of the former type, while *I*, also a flavone factor, was of the latter type. However, in a later study with Scott-Moncrieff (45) it was concluded that the *I* gene was also inherited tetrasomically, but that simplex plants resembled the nulliplex, thus modifying the expected tetrasomic ratios.

It was in Lawrence's first report on *Dahlia* (43) that the phenomenon of double reduction was first suggested.

Rubus. Tetraploid segregation in *Rubus* was reported by Crane and Darlington (15) in connection with the factors for presence and absence of prickles. They found an excess of recessives over those expected according to Muller's hypothesis, which they explained on the assumption that there was a "tendency toward allosyndesis". This explanation was later abandoned (16) and their data were explained on the basis of chromatid segregation, but the

populations were too small to permit any statistical conclusions other than that the deviations from Muller's ratios were significant.

The type of segregation observed in several tetraploid species of *Rubus* has been used by Crane (14) and Thomas (83) to determine whether these species were allo- or autotetraploids.

Maize. Randolph (70), in a report on the cytogenetics of tetraploid *Zea mays*, states that segregations approximating 35:1 ratios were noted for several factors. However, he says that the "data are inadequate to determine whether or not there are significant deviations from the expected ratios".

Lotus. An interesting case of tetraploid segregation in *Lotus corniculatus* was reported by Dawson (22). This species is tetraploid, and two genetic types are found in natural populations, differing only in that one produces hydrogen cyanide and the other does not. It was found that this cyanogenetic character gave ratios which fit very closely those of Muller's random chromosome segregation, and it was therefore concluded that this species is an autotetraploid. The cytological observations gave no evidence of its autotetraploid nature, since formation of quadrivalents was very rare, a condition which is usually assumed to indicate allotetraploidy. The failure of quadrivalent formation would account for the lack of double reduction found in the observed ratios.

Lythrum. The anomalous behavior of heterostyly in *Lythrum salicaria* has been the subject of controversy since first described by Barlow (2) over thirty years ago. East (25, 26, 27, 28) attempted to explain his observations on the basis of linked duplicate factors, both of which were lethal in the homozygous dominant state. However, the breeding behavior of the mid-style form observed by Fisher and Mather (32, 33, 34, 35) could not be explained by East's hypothesis, and their results were explained on a polysomic basis. The chromosome number of this species is not definitely known, since different numbers have been reported by various workers. Nevertheless, it is known that the species is polyploid in relation to the basic number of the genus, and there are indications that it is numerically a hexaploid. So far it has not been possible to determine whether the inheritance of mid style is tetrasomic or hexasomic, but further work is being carried out to determine this point. Evidence of double reduction was obtained in the segregations for this factor, and a rough estimate of the index of double reduction was calculated by Fisher and Mather (35).

Antirrhinum. In some unpublished work by the writer (52), the segregation of four genetic factors was studied in colchicine-induced tetraploids of the snapdragon, *Antirrhinum majus*. The diploid behavior of these four factors had been studied by Baur (3, 4) and Onslow (65, 66) and found to be independently inherited. Moreover, three of the factors could be read in the seedling stage, so that it was possible to grow and classify very large populations. In the tetraploids it was found that the segregation of each of these four factors was different, depending upon the amount of double reduction due to crossing-over between the gene and the centromere. It was possible to show that the segregation of all factors gave a significant excess of recessives over the expectancies based on the Muller random chromosome hypothesis. Furthermore, the ratios obtained with the anthocyanin-modifying factor (*A*) also differed significantly from the Haldane random chromatid ratios.

Hybrids between tetraploids of different varieties within this species have been referred to by Sparrow, Ruttle, and Nebel (78, 79) as allotetraploids, because they are usually more fertile than intra-varietal tetraploids. Cytological observations, however, did not reveal any marked differences in meiotic behavior between inter- and intra-varietal tetraploids. These observations, along with the fact that genetic factors show typical autotetraploid segregations, would seem to indicate that increased fertility found in some inter-varietal tetraploids is due to genetic factors for fertility rather than to selective pairing which ordinarily distinguished allotetraploids from autotetraploids.

DISCUSSION

Conditions Necessary for Genetic Analysis of Tetraploids

In spite of the fact that a large number of tetraploids have been discovered or produced artificially, many of them are not amenable to genetic analysis, due chiefly to three difficulties commonly encountered. The first and most common of these difficulties is the high degree of sterility often found in autotetraploids. The very nature of tetraploid segregation requires that very large populations be grown and analyzed in order to obtain data that will have any statistical significance. In general, larger populations are required in order to draw valid conclusions in tetraploid material than in diploids. The high degree of sterility of many tetraploids makes it

impossible or at least extremely difficult to secure such large populations.

The second difficulty, curiously enough, is most often found when the first difficulty is absent. It consists of the amphidiploid-like behavior found in some tetraploids. It is now generally recognized that "autotetraploidy" and "amphidiploidy" are rather arbitrary terms and that many tetraploids are intermediate in their behavior so that the distinction is not always clear-cut. Amphidiploidy is not confined to species hybrids, but may arise in autotetraploids through structural changes in the chromosomes or some other form of chromosome differentiation. Increased fertility and decreased segregation are simultaneous consequences of a change from autotetraploidy to amphidiploidy, so that many of the most fertile tetraploids display little or no segregation.

The third difficulty is the lack, in many species, of clear-cut genetic characters which lend themselves readily to classification and analysis. A prominent example of this is found in species of *Lilium*. Nearly all genetic factors which distinguish varieties within a species, such as *L. longiflorum*, are quantitative in nature, and a study of their inheritance would be extremely difficult even in diploids. Another example is that of scab-resistance in the potato, which Krantz and Eide (42) found very difficult to classify with any high degree of precision.

Cytological Behavior and Tetraploid Segregation

As stated previously, cytological behavior has a more pronounced effect on genetic segregation in tetraploids than in diploids. There are three main variables in cytological behavior which may affect the proportion of recessives found in a segregating progeny. These will be discussed in the following order: first, mode of pairing; second, quadrivalent formation; and third, chiasma frequency.

Pairing in a tetraploid may be classified as either random or selective with reference to any given group of four homologous chromosomes. Even within the same tetraploid, some members of a genom may undergo random pairing, while others may display varying degrees of selective pairing. In random pairing, any one of the four homologous chromosomes may pair with any one of the other three with equal probability. The hypotheses of Muller, Haldane, and Mather all assume random pairing, but Gregory's hypothesis assumes a special type of selective pairing.

Selective pairing occurs when the four homologs are not equally homologous but tend to fall into two groups such that the two chromosomes within a group display more affinity than two chromosomes from different groups. In other words, they possess differential affinity. Homology is a relative term and can vary from absolute identity, such as is found in doubled haploids, to the very weak homology found in secondary pairing. As a result the pairing in tetraploids can vary from completely random where the four chromosomes are equally homologous, to completely selective where the plant behaves functionally as a diploid. All degrees of selective pairing between these two extremes may exist. To illustrate how selective pairing may affect segregation in autotetraploids, let us assume that we have a duplex hybrid with the constitution $AAaa$. Furthermore, let us designate the two similar pairs of chromosomes carrying these genes with subscript 1 and 2. Then in a plant with the constitution $A_1A_1a_2a_2$, if selective pairing is complete, there will be no segregation, since only gametes with the constitution A_1a_2 can be formed, and these will unite to form progeny with the constitution $A_1A_1a_2a_2$. If selective pairing is not complete, so that A_1 occasionally pairs with a_2 , then gametes can be produced with the constitution A_1A_1 or a_2a_2 , and the latter type may unite to give a homozygous recessive segregate. This is known to occur in *Primula kewensis* (63, 67) where a limited amount of segregation takes place. It was also reported by Skalinska (76) in an allotetraploid specimen of *Aquilegia*. Chromosome doubling in a diploid hybrid A_1a_2 , or crossing of two autotetraploids $A_1A_1A_1A_1$ and $a_2a_2a_2a_2$, will give rise to amphidiploids with the constitution $A_1A_1a_2a_2$ just discussed. However, under some conditions we may have tetraploids with the constitution $A_1a_1A_2a_2$. In this case, complete selective pairing will produce gametes with the constitution A_1A_2 , A_1a_2 , a_1A_2 , and a_1a_2 in equal proportions, and the phenotypic ratios on selfing will be 15A:1a. Moreover, under these conditions two types of duplex hybrids will be produced, those like the parent and non-segregating types $A_1A_1a_2a_2$ or $a_1a_1A_2A_2$. This type of segregation is found in diploids when duplicate factors are involved, and its occurrence is sometimes considered an indication of tetraploid ancestry. We have already pointed out that newly arisen tetraploid hybrids are almost certain to have the constitution $A_1A_1a_2a_2$. The origin of the second type, $A_1a_1A_2a_2$, is probably secondary, re-

sulting from the occasional pairing and consequent crossing-over between A_1 and a_2 chromosomes in such a way that there is an exchange of alleles A and a without conspicuously altering the original differentiation of the chromosomes. This is borne out by the fact that practically all cases of duplicate factor ratios are found in plants that, on other grounds, show evidence of being old polyploids.

Gregory's hypothesis was based on the assumption that there was complete selective pairing between chromosomes from opposite parents. This type of pairing was later referred to as "allosyndesis", and Crane and Darlington (15) spoke of deviations from the 35:1 ratio in *Rubus* as due to a "tendency toward allosyndesis", an explanation which they later retracted (16). The terms "autsyndesis" and "allosyndesis" have been purposely avoided in the foregoing discussion to avoid the confusion that is common in their usage. Autosyndesis is generally defined as pairing between chromosomes derived from the same parent, and allosyndesis as pairing between chromosomes from opposite parents. However, it makes a difference whether we refer to the original parents or to the immediate parents. Darlington (21, p. 200) states: "The distinction between the two is only relative to the immediate parents . . .". He further restricts the use of the term to "pairing of dissimilar chromosomes". Hence he says: "Autosyndesis occurs in allopolyploids under the following conditions: In the normal polyploid species as an exceptional occurrence". Cadman (12), on the contrary, speaks of "duplex allotetraploids . . . where strict autosyndesis prevails". Unlike Darlington, he is referring to autosyndesis as pairing between chromosomes from one of the *original* parents of the allotetraploid. Sharp (74, 75) uses the terms in this latter sense, although he refers to them as "auto-" and "allosynapsis". The terms are used in still another sense in the literature when they refer to genetic differences. Thus in a duplex hybrid $AAaa$, pairing between A and A or a and a is sometimes called "autosyndesis", while pairing between A and a is called "allosyndesis". Lindstrom (51) has used the terms in this genetic sense.

It appears that selective pairing is rare or entirely absent in most of the recently originated autotetraploids that have been studied genetically, such as *Primula sinensis*, *Datura*, *Lycopersicon esculentum* and *Antirrhinum*. The genetic ratios obtained in these plants are therefore not affected by this cytological variable. However, in

old polyploids such as potato and *Dahlia*, there is apparently some selective pairing which tends to alter the segregation of certain genetic factors.

The second cytological variable that affects segregation is the formation of quadrivalents. Quadrivalent formation involves pairing among all four homologous chromosomes. Hence, Gregory's ratio, based on pairing only between chromosomes of opposite parents, could not be obtained where quadrivalent formation is common. Muller's ratios, on the other hand, are not affected by the presence or absence of quadrivalents as long as crossing-over does not occur between the gene and the centromere. In fact, his ratios can be obtained in the complete absence of quadrivalent formation. This appears to be the case in *Lotus corniculatus* (22). Haldane's ratios can not be obtained unless the chromosomes involved form quadrivalents regularly. In Mather's formulae, the quantity a , representing the amount of genetical non-disjunction, is dependent upon the amount of quadrivalent formation, attaining its random value of $1/3$ only if quadrivalents are regularly formed.

The third cytological variable is the frequency and position of chiasmata formation. Since separation at first division is always reductional at the centromere, equational separation at a given locus depends upon the formation of a chiasma between that locus and the centromere. In Mather's formulae, the amount of equational separation is designated as e . If one chiasma is always formed between the centromere and the locus in question, separation at that locus is always equational and e has the value of 1. Stated in terms of cross-over percentage, this would be equivalent to fifty per cent crossing-over, and in general terms, e is twice the cross-over percentage. Many *Drosophila* workers have shown that temperature changes and age affect the amount of crossing-over (10, 68, 69, 80). Ernst (31) has shown that sudden changes in temperature reduce the chiasma frequency and hence the amount of crossing-over in *Antirrhinum*. The subject of environmental effects on chiasma formation has been reviewed by Oehlkers (64). Since the segregation in a tetraploid varies with the amount of chiasma formation, and this is in turn influenced by environmental factors, tetraploid ratios should be expected to vary according to the environmental conditions which prevailed when the parent organism was undergoing meiosis. This has never been demonstrated, but would be well worth investigation.

In a tetraploid, when the two chromosomes in a gamete are derived from sister chromatids, double reduction is said to have occurred. Since double reduction tends to increase the proportion of homozygous gametes (both recessive and dominant), it affects the genetic ratios by increasing the proportion of recessive phenotypes above that expected on the assumption that the chromosomes behave as units and do not undergo double reduction. Hence, any cytological behavior that favors double reduction tends to increase the proportion of recessives in a ratio. In order for double reduction to take place, a special set of cytological conditions must be satisfied. First, paired chromosomes bearing opposite alleles must pass to the same pole at the first division. This is termed "genetic non-disjunction" and its frequency was designated by Mather with the symbol a . Genetic non-disjunction is in turn dependent upon the formation of trivalents or quadrivalents, since with bivalents the two members of the pair almost invariably pass to opposite poles. The second condition which must be fulfilled is that at least one chiasma must be formed between the centromere and the locus of the gene in question, resulting in equational separation of the gene loci at the first division. When both of these conditions are fulfilled, two chromosomes pass to the same pole at the first division, each consisting of two chromatids bearing opposite alleles. At the second division, when the centromeres divide and pass to opposite poles, the two chromatids bearing the same allele may pass to the same pole, thus resulting in double reduction. Inasmuch as double reduction depends upon these two conditions, its frequency may be expressed as the product of the frequency of genetic non-disjunction a , and the frequency of equational separation e . This product ae is designated by Mather as *alpha*.

The index *alpha* gives us the product of the amount of genetical non-disjunction and the amount of equational separation, but tells us nothing about the relative part that each of these variables plays in affecting segregation. The value of a will vary from zero, when no quadrivalents are formed, to $1/3$, the random value obtained when the chromosomes always associate in quadrivalents. The value e , on the other hand, can vary from zero to one as the cross-over percentage between the gene and the centromere varies from zero to fifty per cent. Thus the value of *alpha* should vary from zero to 0.33. If we observe cytologically that the frequency of

quadrivalents is very high, we can assume that the value of a approaches its upper limit of $1/3$, and as a result gain an approximation of e by multiplying the value of α by three.

We have seen what relationship exists between tetraploid segregation and several specific aspects of cytological behavior. It should also be pointed out that segregation in tetraploids has an important bearing on several general cytological concepts. One of these is in regard to the time of crossing-over. Deviations from the Muller ratios furnish us with genetic proof that crossing-over takes place in the four-strand stage between two of the strands. The first demonstration of this fact was furnished by Anderson (1) in connection with studies on crossing-over in attached X-chromosomes of *Drosophila*. It was further demonstrated by Bridges and Anderson (11) in the X-chromosomes of triploid females of *Drosophila*. It is an interesting fact that the data on tetraploid segregation published previously by Blakeslee, Belling, and Farnham (9) can now be interpreted to prove the same fact. The appearance of recessive progeny from triplex hybrids could not have taken place without double reduction and hence crossing-over in the four-strand stage. The first genetic demonstration of double-strand crossing-over in *Zea* was made by Rhoades (71, 72) in work based on the genotypic constitution of trisomic types. Emerson and Rhoades (30) have pointed out the significance of chromatid or double-strand crossing-over in relation to the upper limit of recombination. Finally, the ingenious work of Lindegren on *Neurospora* (48, 49) proved the occurrence of double-strand crossing-over by an entirely different method and shed light on further details in the behavior of chromatids and chromosomes during meiosis.

Another general problem, upon which tetraploid segregation can throw light, is the old problem of reductional and equational separation of chromosomes during meiosis. It was long thought that the first division of meiosis was "reductional" or "heterotypic" while the second division was "equational" or "homotypic". This distinction is now known to apply only to the centromere and closely adjacent regions of the chromosomes, for the other portions of the chromosomes may divide equationally at the first division as the result of crossing-over. As a result of these "equational exceptions", double reduction can occur, a fact first suggested by Lawrence (43) and further discussed by Darlington (20) in 1929. This problem has been discussed in detail by Mather (57, 59).

Genetical Behavior and Tetraploid Segregation

Not only does tetraploid segregation have a close relation to various cytological problems, but it is also of interest in connection with certain genetical problems. The foremost of these is the location of the centromere on the linkage map of a chromosome. It is impossible to solve this problem in the study of diploid ratios (except in the fungi), but a solution can be approximated if we have autotetraploids from which to secure data on segregation. This method was used by De Winton and Haldane (24) to determine the approximate position of the centromere on the linkage map of one of the chromosomes of *Primula sinensis*. As already pointed out, there is no method of determining the amount of crossing-over between the gene and the centromere with any high degree of accuracy, because two cytological variables are involved. However, we can obtain relative values, so that with chromosomes on which we have a large number of markers it should be possible to locate the centromere within rather close limits.

There is one difficulty which is apt to be encountered rather commonly in connection with interpretation of ratios. This is the problem of differential viability of various phenotypes. A great many recessive genes tend to reduce viability, and when a tetraploid is segregating for such a character, the introduction of another unknown variable renders the interpretation of results almost impossible. It is true that we can sometimes calculate the extent of differential viability in a diploid, and make corresponding allowances in calculating tetraploid expectancies. However, it is scarcely safe to assume that the extent of viability will be the same in the tetraploid as in the diploid. As a matter of fact, there is considerable evidence, especially in maize, that the deleterious genes accumulated in inbred diploid lines have a still more deleterious effect in the autotetraploid plants from these lines even though the change is one of quantity and not of proportion. Therefore, any results obtained from the study of tetraploid segregations that involve characters showing differential viability should be regarded as only very rough approximations.

Differential viability has an interesting effect on the values of *alpha* obtained from progenies of duplex and simplex hybrids. Since the proportion of recessives is usually decreased by differential viability, there is a tendency for the value of *alpha* to be decreased below what its value would be if the dominants and recessives were equally viable. However, the amount of decrease is not the same

for progenies of duplex hybrids as it is for progenies of simplex hybrids. For example, where double reduction is at a maximum, the value of α is 0.333, but differential viability eliminating 18.7% of the recessives is sufficient to change the value of α to zero in progenies from a simplex hybrid. On the other hand, in progenies from a duplex hybrid, the same amount of differential viability will reduce the value of α only to 0.208. In general, the same amount of differential viability will reduce the value of α in simplex progenies to a greater extent than it will in duplex progenies. Mather (59) noted just such differences in the progenies from duplex and simplex hybrids and postulated two different constants, α for simplex and triplex segregations, and β for duplex segregations. He explained the difference on the grounds that changes of partner in pachytene pairing would affect the two types of segregations differently. The writer, in an unpublished report (52), suggested that this cytological explanation would not account for the difference between constants, and that differential viability appeared to be the most likely explanation. Recently, Mather (35) has acknowledged the incorrectness of his original explanation and suggested three other explanations, namely: chromosome differentiation, misclassification, and disturbed viability. We have already seen that chromosome differentiation tends to reduce the amount of segregation, and hence the value of α , in progenies from duplex hybrids, whereas it will have little or no effect on segregation in simplex progenies. Therefore, chromosome differentiation should produce an effect just the opposite of that which has been observed. It is also difficult to see how misclassification could explain the observed differences in α between simplex and duplex progenies. It appears likely that the most common source of error in classification would lie in the simplex plants of a progeny, since these are often similar to the nulliplex and might frequently be classified as such. Such an error in classification would increase the value of α above its true value, but the value of α from simplex progenies would be increased to a greater extent than the value from duplex progenies. Here again, the observed effect is the opposite from the one expected according to this explanation. As we have seen, differential viability would produce a result in accordance with observations, namely a value of α from simplex progenies less than that from duplex progenies. It is the only explanation yet proposed which readily accounts for the observed facts, and an explana-

tion which does not appear unreasonable, since we are dealing with a very common phenomenon.

In regard to the phenotypic results produced by genes, several unique phenomena are found in tetraploids. Whereas in diploids there are only three possible genotypes involving a pair of alleles, in tetraploids there are five. In two of these genotypes, the triplex and simplex, the proportion of dominant to recessive genes differs from the equal proportions always found in diploid hybrids. Often when two alleles are present in equal proportions, one is completely dominant over the other, but it may not be capable of completely masking the effect of three recessive genes in a simplex hybrid. For this reason, incomplete dominance is more common in tetraploids than in diploids. In *Primula*, for example, Sömme (77) has shown that in diploids the factor *G* for green stigma is completely dominant over the gene *g* for red stigma, affecting not only stigma color but also suppressing color in the flowers. In tetraploids, on the other hand, plants with the constitution *Gggg* have green stigmas, but "the flowers have a darker shade, in some cases nearly as dark as that of the pure recessive form". In addition, the factor *B* for magenta flower color is completely dominant over red in the diploid, but in the tetraploid the class *Bbbb* "exhibits colours varying from magenta to almost pure red". An even greater increase of incomplete dominance was found in connection with interactions involving the dominant white gene, *W*, and the green stigma factor, *G*. In tetraploids, the number of combinations of genotypes is so increased that they "may, according to their coloring, be arranged in an almost continuous series, ranging from pure white through lighter and darker shades to pure magenta or red". Sansome (73) has reported similar cases in tomato, where one dominant gene is not completely dominant over three recessives. In *Antirrhinum* (52) the basic color factor *B* and the magenta anthocyanin factor *A* appear to be completely dominant even in the simplex condition. However, the ivory flavone factor *C* will not completely suppress the formation of yellow flavone in simplex plants, so that *Cccc* plants have considerably more yellow color than those containing two or more dominant *C* genes. It was also found that the basic anthocyanin factor *F* produced an almost continuous gradation of colors from full to very dilute as the number of recessive genes increased from zero to three.

One of the most striking genetic effects accompanying a change from diploidy to autotetraploidy is in connection with self-incompati-

bility. Lewis and Modlibowska (46, 47) have concluded that all natural autotetraploid forms of self-incompatible species are self-compatible. However, this conclusion is too broad, for there are some exceptions, such as the North Temperate autotetraploid species of *Tradescantia*, which are as self-incompatible as the diploids from which they arose. Some but not all of the experimentally produced autotetraploids of self-incompatible plants are self-compatible. The complex physiological mechanism, controlled by the *S* genes in diploids, is apparently upset in tetraploids so that the inhibition of pollen tube growth is in some way weakened. Where inhibition of pollen tube growth is rather weak in the diploid, it may disappear altogether in the tetraploid. Such seems to be the case in *Petunia*, as reported by Stout and Chandler (82), where the tetraploids were completely self-compatible. In *Oenothera organensis*, on the other hand, Lewis (46) has shown that inhibition of pollen tube growth is very strong in the diploid, and while it is reduced in the tetraploid, the reduction is not sufficient to allow fertilization to take place. In the cultivated apples, pears, and stone fruits, self-compatibility has been found much more common among polyploid forms than among the diploids (17, 18, 19, 47), but the genetic mechanism for incompatibility is so complex that a full explanation of the findings has not been possible. Howard (39) found that polyploid forms of *Brassica* and *Raphanus* are as self-incompatible as the diploids, but in this case, self-incompatibility is apparently not determined by oppositional alleles as in most self-incompatible plants.

Statistical Analysis of Tetraploid Segregation

The unfixed nature of tetraploid ratios introduces problems in statistical analysis not encountered in dealing with fixed diploid ratios. It is rather common in the literature for geneticists to refer to "chromosome segregation" and "chromatid segregation" as though these were two fixed ratios between which one must choose in fitting a set of data. As a matter of fact, these two types of segregation are, in reality, only ideal limiting ratios which are seldom attained, and probability favors the occurrence of ratios which are intermediate between these two limits.¹ The important statistical

¹ As Mather (57) pointed out, random chromatid segregation is really not the upper limit as far as proportion of recessives is concerned. Data may simulate the random chromatid ratios due to the balance between 6/7 equational and 1/7 reductional separation. Complete equational separation at a locus will produce an even higher proportion of recessives.

problem is, therefore, not to determine which ratio the data fit more closely, but to find out to what extent the two opposing forces of reductional and equational separation have affected the data. Mather's index, α , conveniently characterizes a set of data in this regard.

It has been shown that the data on *Datura* published by Blakeslee, Belling, and Farnham (9) differed significantly from either the Muller or the Haldane ratios. On the other hand, it has been shown that Gregory's data (37) did not differ significantly from either type of ratio. These two examples bring out the point that large populations are necessary in order to draw valid conclusions in regard to tetraploid ratios. For example, it is necessary to have a population of at least 1,700 plants in order to be certain that any ratio obtained can be proven to deviate significantly from either a 35:1 or a 21:1 ratio.

The method of calculating expected ratios when the value of α is given is merely one of substituting this value in the formulae in Table I, column 5. The converse problem of calculating the value of α from observed data is discussed fully by Mather (57, 58, 59). Briefly the method consists of setting up a log likelihood equation in which the likelihood is expressed as the sum of the products of the observed number in each class and the log of the expectancy in terms of α . This expression is then differentiated with respect to α , and the derivative equated to zero. The solution of this equation is then the value of α giving the "maximum likelihood". The variance is then obtained by taking the second derivative of the original equation, substituting the value of α , and taking the negative reciprocal of the result. When data are available on several types of matings, the maximum likelihood equations will be rather complex and must be solved by successive approximations. When we have only data on progenies from selfed plants, as is often the case, the equations reduce to very simple forms. Thus, to calculate α from the progeny of a selfed simplex plant, we can use the equation: $\alpha = 8\sqrt{z} - 4$, where z represents the percentage of recessives found in the population. The standard error will be $4\sqrt{(1-z)/n}$. For data from a selfed triplex plant, the value of α is $8\sqrt{z}$, and the standard error is the same as in the last case. The value of α derived from the progeny of a selfed duplex plant can be calculated from the formula: $6\sqrt{z-1}$, and the standard error will be $3\sqrt{(1-z)/n}$.

Practical Significance of Tetraploid Segregation

Several of our horticultural crops are autotetraploid or behave partly as autotetraploids, and in recent years autotetraploids have been produced artificially among a wide variety of economic plants. For these reasons the problems of autotetraploid genetics are of considerable practical importance to the plant breeder. As an example let us consider the common problem in plant breeding of obtaining a true-breeding line. In diploids this is relatively simple, for when a selection is made in an F_2 or later generation which produces a uniform progeny, all plants in such a progeny are concluded to be homozygous, and one feels safe in bulking the seed and using it for an increase. Such procedure would be decidedly unsafe in an autotetraploid, for the selected plant may be triplex. In this case its progeny would be phenotypically uniform, but genotypically it would still be segregating, producing some duplex and even simplex plants which would segregate in the following generation. Just such a problem has been pointed out by Cadman (12) in connection with obtaining a strain of potatoes homozygous for resistance to virus.

Another problem which arises is the difficulty of obtaining multiple recessive types from a cross. As a simple example, suppose that a cross is made between two parents which differ by only three genetic factors, in an attempt to secure a plant recessive for all three. In a diploid the probability of obtaining such a triple recessive is 1 in 64. In an autotetraploid, however, the probability ranges from 1 in 8,000 to 1 in 46,656, depending on the amount of double reduction operating to increase the proportion of recessives. Obviously one must either grow very large populations or devise some method of obtaining the desired type in several steps.

Besides being of practical significance to the plant breeder, the study of autotetraploid genetics has another more indirect value, which consists of furnishing an approach to the study of quantitative inheritance. This problem has been one of the most baffling and complex encountered in the field of genetics, and is still far from being solved. Yet it is of the utmost economic importance, since a great majority of the genes which contribute to the value of economic plants are quantitative in character, and a thorough understanding of their hereditary behavior is greatly to be desired. Lindstrom (50) states that "comparison of quantitative inheritance in $2n$ and

4n affords another approach to the problem of quantitative inheritance", and he has taken the first step in this direction through a study of quantitative genes in diploid and tetraploid tomatoes. With the increase of tetraploid plants available for study, further work along this line should be possible and should prove highly profitable.

SUMMARY

Three main theories of autotetraploid segregation have been propounded, the Muller hypothesis based on the random assortment of chromosomes at meiosis, the Haldane hypothesis based on the random assortment of chromatids, and the Mather hypothesis in which the ratios are considered to be not fixed but varying according to the amount of quadrivalent formation and the distance of a gene from the centromere. The first two hypotheses are special cases of the last, which is, in general, the most satisfactory for explaining observed data.

While many autotetraploids do not lend themselves readily to genetic analysis, due to sterility, chromosome differentiation or lack of good genetic characters, fairly extensive studies have been carried out on the autotetraploid genetics of ten species in as many genera.

The cytological variables that affect autotetraploid segregation are mode of pairing, formation of quadrivalents, and number and position of chiasmata. In progenies unaffected by variables other than these, it is possible to gain an estimate of the position of the gene involved with respect to the centromere.

Differential viability markedly affects some of the autotetraploid ratios, and can account for some of the discrepancies between observed data and expectancies.

Incomplete dominance is a more common phenomenon among tetraploids than among diploids, due to the greater number of genotypes possible for any given pair of factors.

On account of striking differences between the genetics of diploids and tetraploids, plant breeding procedures applicable to diploids must frequently be modified in dealing with tetraploids.

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Destructive Foreign Plant Diseases not known to be established in North America	N. REX HUNT <i>Bureau of Entomology and Plant Quarantine</i>
Cytology and Genetics in Relation to Taxonomy ..	C. L. HUSKINS <i>McGill University</i>
Techniques for Aseptic Growth of Plants	L. C. KNORR <i>Cornell University</i>
Protoplasmic Connections	L. G. LIVINGSTON <i>Harvard University</i>
Endomitosis	A. LORZ <i>Seton Hall College</i>
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Cell-shape	E. B. MATZKE <i>Columbia University</i>
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Plant Disease Introduction	W. A. MCCUBBIN <i>Bureau of Entomology and Plant Quarantine</i>
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Taxonomy of North American Fresh-Water Algae	L. H. TIFFANY <i>Northwestern University</i>
Nematode Diseases of Plants	A. L. TAYLOR <i>Bureau of Plant Industry</i>
Measurement of Soil Moisture	F. J. VEIHMEYER <i>University of California</i>
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Utilization of Native Plants by the American Indians	P. A. VESTAL <i>Harvard University</i>
The St. Lawrence River as a Biological Habitat for Higher Plants	FR. MARIE-VICTORIN <i>University of Montreal</i>
Vegetable Seed Treatment	J. C. WALKER <i>University of Wisconsin</i>
Detached Leaf Culture	C. E. YARWOOD <i>University of California</i>
Modern Views and Facts About Growth Substances	P. W. ZIMMERMAN <i>Boyce Thompson Institute for Plant Research</i>

Proposed Future Contents of THE BOTANICAL REVIEW

Articles received and awaiting publication

Air Space Tissue in Plants	H. B. SIFTON University of Toronto
Plant Growth Relations on Saline and Alkali Soils	O. C. MAGISTAD U. S. Regional Salinity Laboratory
Colchicine and X-rays in the Treatment of Plant and Animal Overgrowths	MICHAEL LEVINE Montefiore Hospital
A Critical Survey of the Present Status of Plant Embryology	DONALD A. JOHANSEN Stanford University
Taraxacum kok-saghit as a Source of Rubber ...	G. KROTKOV Queens University, Canada
Gene Segregation in Autotetraploids	THOMAS M. LITTLE U. S. Bureau of Plant Industry
A Critical Survey of the Present Status of Plant Embryology	D. A. JOHANSEN Stanford University, Cal.
Cytotaxonomy of Nicotiana	T. H. GOODSPEED University of California
Tissue Responses to Physiologically Active Substances	BETTY F. THOMSON Connecticut College
Absorption of Water by Plants	PAUL J. KRAMER Duke University
The Proper Designation of the Vascular Plants...	THEODOR JUST University of Notre Dame

Articles arranged for most recently

Root Rots of Deciduous Fruit Trees	J. S. COOLEY U. S. Department of Agriculture
Heart Rots in Living Trees	R. W. DAVIDSON U. S. Bureau of Plant Industry
Cell Walls	T. KEER North Carolina State College

Articles in course of preparation

The Cytology of Fertilization in Angiosperms	L. E. ANDERSON Duke University
Development of the Madre-Tertiary Flora	D. I. AXELROD University of California
Relation of Wood Anatomy to Taxonomy	I. W. BAILEY Harvard University
Rubber Formation in Plants	JAMES BONNER and A. W. GALSTON California Institute of Technology
Role of the Endosperm in Seed Development	R. A. BEINK and D. C. COOPER University of Wisconsin
Heart Rots in Living Trees	R. W. DAVIDSON Bureau of Plant Industry
The Angiosperm Embryo Sac	EMMA L. FISK University of Wisconsin
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